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OBSERVATIONS ON THE BODY TEMPERATURE OF DRY COWS

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COOPERATIVE INVESTIGATIONS BETWEEN THE INSTITUTE OF ANIMAL NUTRITION OF THE PENNSYLVANIA STATE COLLEGE AND THE BUREAU OF ANIMAL INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE

The normal temperature of the animal body is never a constant figure. It varies in different species, in different individuals, and is never absolutely constant even in the same individual. The observations on the body temperature of man are numerous and more or less conclusive, while relatively little attention seems to have been given to the study of the variations in the body temperature of farm animals under different conditions which are known to affect the temperature of man.[2]

In healthy warm-blooded animals, notably cattle, as well as man, the body temperature fluctuates within very narrow limits. When, however, the normal processes of the body are upset, the temperature may vary widely, and for this reason body temperature is looked upon as an index of health. But aside from this and from any other physiological significance that it might have, knowledge of the normal course of fluctuations in body temperature of cattle has grown in importance with the perfection of the respiration calorimeter used in the investigations on metabolism with cattle.

By means of the respiration calorimeter the heat produced by the animal during a certain period of time, as well as the gaseous exchange between the animal and the atmosphere that surrounds it, is measured. But in order to determine accurately the heat production as distinguished from the heat emission, a knowledge of the storage or loss of heat by the animal body is indispensable. If at the end of an experimental period

[1] I am under obligation to Dr. H. P. Armsby for the opportunity to carry out this investigation, and I wish to thank him for his invaluable suggestions and kindly criticism. I also wish to thank the other members of the staff, especially Prof. J. A. Fries and Prof. W. W. Braman for the interest they have shown and for the suggestions they have given me.

[2] PEMBREY, M. S. ANIMAL HEAT. *In* Schäfer, E. A., ed. Text-Book of Physiology. p. 785–867, fig. 76–82. Edinburgh, London, New York, 1898.

the body temperature of an animal weighing 400 kgm. is 1° C. higher or lower than that at the beginning, it would mean, in the first case, a storage and, in the second case, a loss of heat by the body amounting to 332 calories (assuming the specific heat of the body to be 0.83). Such a quantity would be too large to be entirely ignored in accurate determinations of heat production.

It has been generally claimed that in 24- or 48-hour respiration calorimeter experiments no serious error is introduced by the assumption that the body temperature is approximately the same at approximately the same hour of the day. It was the main purpose of this experiment to obtain data regarding the extent and the course of the body temperature variations in cattle and to study some of the factors that might influence them. With this object in view, observations on the body temperature of cows have been made with reference to the study of the following:

1. Variations in body temperature from about 7.30 a. m. to about 5.30 p. m., including the effect of water drunk.
2. Thermal gradient in the body.
3. Variations in body temperature from about 5 p. m. to about 7 p. m., including the effect of the feed.
4. Effect of the act of defecation and of change in position.
5. Daily variations in temperature measured at exactly the same time of the day.

SUBJECT AND CONDITIONS OF THE EXPERIMENT

It is essential, in order to get comparable results, that the temperature observations on the subject be made under as strictly uniform conditions as practicable. This condition of the experiment fortunately existed to great satisfaction, since the animals used for this experiment were those used at the same time at the Institute of Animal Nutrition for metabolism experiments with the respiration calorimeter, in which strict control of feeding, environment, etc., is maintained. It may perhaps not be superfluous to give a complete description of the animals used for this experiment in order that a fuller appreciation of the individual variations in body temperature may be later had in considering the experimental results.

DESCRIPTION OF THE ANIMALS

The animals used were two dry cows, No. 885 and No. 886. They are both pure-bred registered Jerseys. Cow 885 was born on March 27, 1914, and dropped her first and last calf on September 15, 1917. Cow 886 was born on July 13, 1914, and dropped her first and last calf on October 10 1917. They weigh about 400 kgm. each and resemble each other very much in respect to both size and color.

Both cows were in good health throughout the experiment.

LOCATION

The cows were located during the experiment in the institute barn, which is a closed wooden building provided with a coal stove so that the temperature of the room could be kept fairly uniform.

TIME AND RATIONS

The investigation covers a period of some 16 weeks from December 1, 1919, to March 22, 1920. The cows were kept on a maintenance ration, except that from December 20, 1919, to January 24, 1920, cow 886 received a supermaintenance ration, and from January 10 to February 7, 1920, cow 885 received a supermaintenance ration, about 1.5 times the maintenance ration in both cases.

FEEDING AND WATERING

The cows were fed twice during every 24 hours, at 6 a. m. and at 5 p. m., unless the time was changed for experimental purposes, in which case it has been specified. They were watered only once a day, at about 8 a. m., the amount of water drunk and the temperature of the water being recorded.

BARN TEMPERATURE

A continuous record of the temperature of the barn was obtained by means of the Columbia recording thermometer. But besides this the temperature of the barn, as indicated by a mercurial thermometer, was recorded simultaneously with every observation on the body temperature of the animal.

METHOD OF MAKING BODY-TEMPERATURE OBSERVATIONS

The observations on the body temperature were made by means of carefully standardized clinical thermometers. During the greater part of the experiment one and the same thermometer was used, while for simultaneous readings of the rectal and vaginal temperatures a second one was used. To avoid any possible error as to the relative value of the rectal and vaginal temperatures that might be due to the use of two different thermometers, the latter were for a few days interchanged—that is, the thermometer used one day for measuring the rectal temperature was used the following day for the vaginal, and vice versa. This was further checked by using one and the same thermometer for both the rectal and vaginal temperature readings, taken one immediately after the other.

All observations except those on the thermal gradient were made at a depth of 7 inches. The thermometers, which were originally 5 inches long, were accordingly lengthened by means of a piece of rubber tubing slipped ½ inch or so over the upper end of the thermometer and the

remaining portion over the string attached to the loop of the thermometer and stiffened by inserting inside the rubber tubing a strip of copper wire. This was finally covered with adhesive plaster and a rubber ring tightly fitted at the end. This arrangement proved very satisfactory. In the first place, the thermometer could be inserted to the desired depth, and, in the second place, there was little danger of breaking it during the insertion.

The thermometer was ordinarily allowed to stay in the rectum or vagina for about three minutes, and the time and temperature were recorded immediately upon its removal.

EXPERIMENTAL RESULTS

The results are expressed in the form of curves. In all curves except those of the thermal gradient the hours or dates are expressed by horizontal distances, and the corresponding temperatures are represented vertically. In the curves of the thermal gradient the horizontal dis-

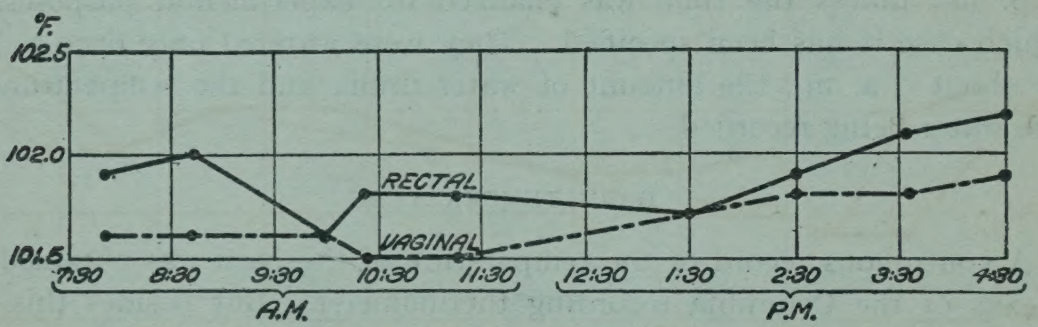

FIG. 1.—Variations in body temperature of cow 886 on December 1, 1919, 7.50 a. m. to 4.30 p. m.; 7.50 a. m. immediately after urinating; 8.25 a. m. drank 5.2 kgm. of water at 52° F.; 10 a. m. lying; 10.22 a. m. standing and immediately after defecating; 11.15 a. m. lying; 1.30 p. m. lying; 2.30 to 4.30 p. m. standing; 3.32 p. m. immediately after defecating.

tances represent the depths of insertion, and the vertical distances represent the corresponding temperatures.

The barn temperatures are not given because they were too uniform to have had any appreciable effect on the body temperature, ranging in most cases between 50° to 60° F.

To facilitate comparisons, the different types of curves for the two cows have been arranged to follow each other in more or less chronological order.

VARIATIONS IN BODY TEMPERATURE FROM ABOUT 7.30 A. M. TO ABOUT 5.30 P. M.

OBSERVATIONS ON DECEMBER 1, 1919, WITH COW 886

Readings of the rectal and vaginal temperatures were taken at approximately 1-hour intervals from 7.50 a. m. to 11.15 a. m. and from 1.30 p. m. to 4.30 p. m. The vaginal temperature was measured immediately after the rectal by the same thermometer. The reading of the vaginal temperature at 7.50 a. m. was taken immediately after the cow had

urinated. At 8.25 a. m. the cow drank 5.2 kgm. of water at 52° F. During the observations at 10 a. m., 11.15 a. m., and 1.30 p. m. the cow was lying; during all others she was standing. The readings at 10.22 a. m. and 3.32 p. m. were taken immediately after the animal had defecated. The results of these observations are shown in figure 1.

The curve for the rectum shows a slight fall in temperature between 8.40 a. m. and 10 a. m., the period following the drinking of the water. It then slightly rises and from 10.22 a. m. to 1.30 p. m. forms almost a straight line. From 1.30 p. m. to 4.30 p. m. it shows a gradual rise of temperature.

The curve for the vagina is in general lower than that for the rectum. It does not show, however, any effect of the water drunk, forming a straight line till 10 a. m. and meeting the rectal curve at that time. The two curves then diverge to meet again at 1.30 p. m. They then diverge again but soon run almost parallel to each other till 4.30 p. m.

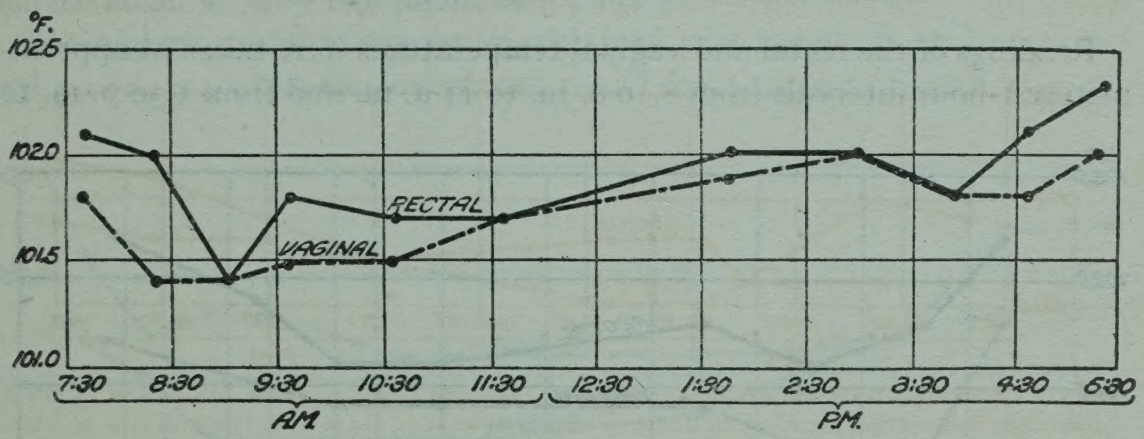

FIG. 2.—Variations in body temperature of cow 886 on December 2, 1919, 7.40 a. m. to 5.20 p. m. At 7.40 a. m. standing; 8.00 a. m. drank 13.0 kgm. of water at 48° F.; 8.20 a. m. lying; 9.00 a. m standing and immediately after defecating; 9.35 a. m. to 10.35 a. m. lying; 2.57 p. m. standing; 3.55 p. m. lying down with thermometers inserted; 4.35 p. m. immediately after defecating; 5 p. m. to 5.20 p. m. eating.

The highest rectal temperature obtained was 102.2° F. at 4.30 p. m., while the lowest was 101.6° at 10 a. m. The highest vaginal temperature obtained was 101.9° at 4.30 p. m., while the lowest was 101.5° at 10.22 a. m. and 11.15 a. m.

OBSERVATIONS ON DECEMBER 2, 1919, WITH COW 886

Readings of the rectal and vaginal temperatures were taken at approximately 1-hour intervals from 7.40 a. m. to 11.35 a. m. and from 1.50 to 5.20 p. m. The rectal and vaginal temperatures were measured at the same time by the two thermometers. At 8 a. m. the cow drank 13 kgm. of water at 48° F. During the observations at 8.20 a. m., 9.35 a. m., and 10.35 a. m. the cow was lying; at 3.55 p. m. the cow lay down with the thermometers inserted. The cow was up during all the other readings. At 9 a. m. and at 4.35 p. m. the readings were taken immediately after defecation. The cow was fed at 5 p. m., and at the end of the observations—that is, at 5.20 p. m.—she was still eating. The results are given in figure 2.

The curve for the rectum, as in figure 1, shows a fall in temperature during the period following the drinking of the water. This drop is, however, more sudden and more noticeable, presumably on account of the greater amount of water drunk. The curve is quite uniform from 9.35 a. m. to 2.57 p. m., when it shows again a fall in temperature till 3.55 p. m., followed by a rise till 5.20 p. m.

The curve for the vagina is again lower than that for the rectum. It shows the effect of the water a little earlier than the rectal. During their course the two curves meet four times. There is, in general, however, some resemblance between figure 1 and figure 2.

The highest rectal temperature obtained was 102.3° F. at 5.20 p. m., while the lowest was 101.4° at 9 a. m. The highest vaginal temperature obtained was 102° at 5.20 p. m., while the lowest was 101.4° at 8.20 a. m. and at 9 a. m.

OBSERVATIONS ON DECEMBER 3, 1919, WITH COW 886

Readings of the rectal and vaginal temperatures were taken at approximately 1-hour intervals from 8.10 a. m. to 11 a. m. and from 1.30 p. m. to

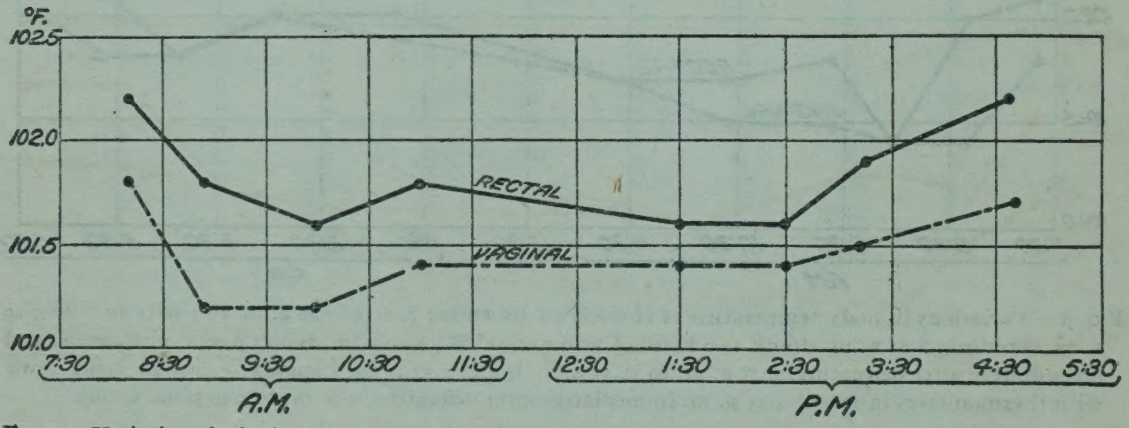

FIG. 3.—Variations in body temperature of cow 886 on December 3, 1919, 8.10 a. m. to 4.36 p. m. At 8 a. m. drank 13.0 kgm. of water at 55° F.; 8.10 a. m. to 8.54 a. m. standing; 10 a. m. immediately after getting up and defecating; 11 a. m. lying; 1.30 p. m. to 4.46 p. m. standing; 3.15 p. m. immediately after defecating; 4.36 p. m. immediately after defecating.

4.36 p. m. The rectal and vaginal temperatures were measured simultaneously by means of the two thermometers interchanged—that is, the one used on December 2 for the rectum was used here for the vagina, and vice versa. At 8 a. m. the cow drank 13 kgm. of water at 55° F. At 11 a. m. the cow was lying. During all other observations the cow was up. The readings at 10 a. m., 3.15 p. m., and 4.36 p. m. were taken immediately after defecation. The results of these observations are given in figure 3.

The curves show the usual fall in temperature after watering and the rise in temperature in the afternoon. The curve for the vagina is lower than that for the rectum and is to a great degree parallel with it.

The highest rectal temperature obtained was 102.2° F. at 8.10 a. m. and 4.36 p. m., while the lowest was 101.6° at 10 a. m. and 2.30 p. m. The

highest vaginal temperature obtained was 101.8° at 8.10 a. m., while the lowest was 101.2° at 8.54 a. m. and at 10 a. m.

OBSERVATIONS ON DECEMBER 4, 1919, WITH COW 886

Observations on the rectal and vaginal temperatures were made at approximately 1-hour intervals from 7.45 a. m. to 5.24 p. m. . The rectal and vaginal temperatures were measured simultaneously with the two thermometers, as on the preceding day. At 8.05 a. m. the cow drank 9 kgm. of water at 54° F. During the readings at 8.57 a. m. and 2.24 p. m. the cow was lying, while during all others she was standing. At 9.33 a. m. the reading was taken immediately after defecation. From 5 p. m. to 5.24 p. m. the cow was eating. The results are represented by figure 4.

The curves show the usual fall in temperature after watering and the rise in the afternoon. The vaginal curve is lower than the rectal, meeting the latter at only one point, at 3.30 p. m.

The highest rectal temperature obtained was 102.2° F. at 4.24 p. m. and 5.24 p. m., while the lowest was 101.6° from 10.42 a. m. to 2.24 p. m.

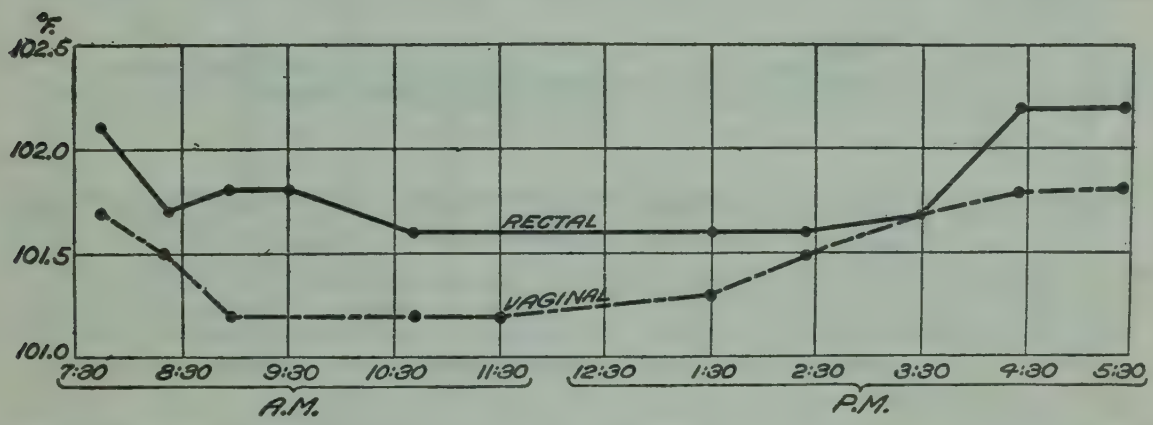

FIG. 4.—Variations in body temperature of cow 886 on December 4, 1919, 7.45 a. m. to 5.24 p. m. From 7.45 a. m. to 8.24 a. m. standing; 8.05 a. m. drank 9 kgm. of water at 54° F.; 8.57 a. m. lying; 9.33 a. m. immediately after getting up and defecating; 10.42 a. m. to 1.30 p. m. standing; 2.24 p. m. lying; 3.30 p. m. to 5.24 p. m. standing; 5 p. m. to 5.24 p. m. eating.

The highest vaginal temperature obtained was 101.8° at 4.24 p. m. and 5.24 p. m., while the lowest was 101.2° from 8.57 a. m. to 11.30 a. m.

OBSERVATIONS ON DECEMBER 5, 1919, WITH COW 886

Observations on the rectal and vaginal temperatures were made at approximately 1-hour intervals from 7.45 a. m. to 11.24 a. m. and from 1.36 p. m. to 5.24 p. m. The rectal and vaginal temperatures were measured at the same time with the two thermometers as on December 2 (fig. 2). At 8 a. m. the cow drank 6 kgm. of water at 54° F. At 7.45 a. m., 9.42 a. m., 11.24 a. m., and 1.36 p. m. the cow was lying; during the other readings she was standing. At 10.36 a. m. and at 3.18 p. m. the readings were taken immediately after defecation. From 5 p. m. to 5.24 p. m. the cow was eating. The results are represented by figure 5.

The peculiar feature of these curves is that the vaginal curve crosses the rectal at two points, besides meeting at another. The usual fall in temperature after watering and the rise in the afternoon are also shown.

The highest rectal temperature obtained was 102.3° F. at 7.45 a. m., while the lowest was 101.45° at 3.18 p. m. The highest vaginal temperature obtained was 102° at 7.45 a. m. and 5.24 p. m., while the lowest was 101.4° at 8.27 a. m., 10.36 a. m., and 1.36 p. m.

OBSERVATIONS ON DECEMBER 1, 1919, WITH COW 885

Observations on the rectal and vaginal temperatures were made at approximately 1 hour intervals from 8.06 a. m. to 11.30 a. m. and from 1.30 p. m. to 4.30 p. m. The vaginal temperature was measured immediately after the rectal by the same thermometer. At 8.30 a. m. the cow drank 29.4 kgm. of water at 52° F. The cow was standing during

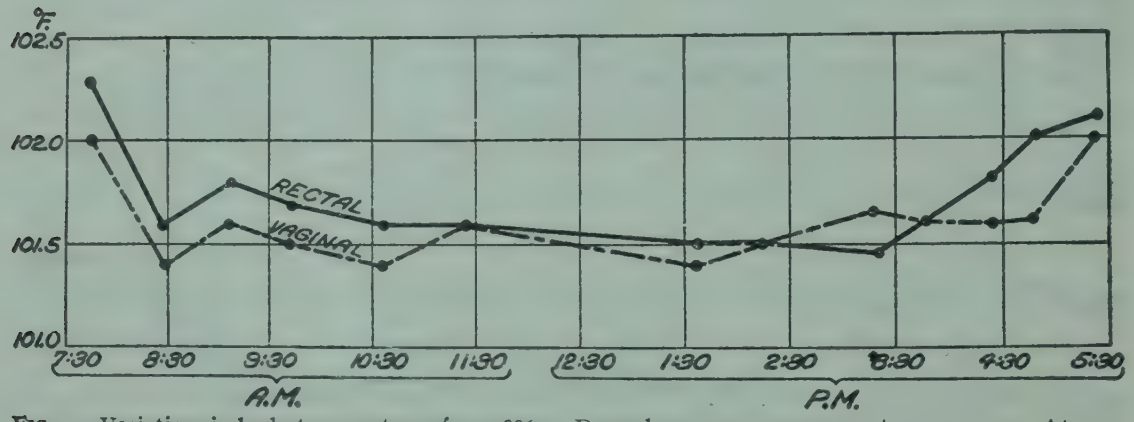

FIG. 5.—Variations in body temperature of cow 886 on December 5, 1919, 7.45 a. m. to 5.24 p. m. At 7.45 a. m. lying; 8 a. m. drank 6 kgm. of water at 54° F.; 8.27 a. m. standing; 9.06 a. m. and 9.42 a. m. lying; 10.36 a. m. immediately after getting up and defecating; 11.24 a. m. to 1.36 p. m. lying; 2.15 p. m. standing; 3.18 p. m. immediately after getting up and defecating; 3.42 p. m. to 5.24 p. m. standing; 5 p. m to 5.24 p. m. eating.

all readings. At 8.12 a. m., 10.12 a. m., and 2.30 p. m. the readings were taken immediately after defecation. The results are represented by figure 6.

The peculiar feature of these curves is the very marked effect of the large quantity of water drunk. The vaginal is lower than the rectal curve and is almost parallel with it, meeting it, however, at two points. The usual rise in temperature in the afternoon is also shown.

The highest rectal temperature obtained was 102.2° F. at 4.30 p. m., while the lowest was 100.2° at 10.12 a. m. The highest vaginal temperature obtained was 101.7° at 8.12 a. m., while the lowest was 100.2° at 10.12 a. m.

OBSERVATIONS ON DECEMBER 2, 1919, WITH COW 885

Readings of the rectal and vaginal temperatures were taken at approximately 1-hour intervals from 7.48 a. m. to 10.45 a. m. and from 2 p. m. to 5.30 p. m. The rectal and vaginal temperatures were measured at the same time with the two thermometers as for figures 2 and 5. At

about 8 a. m. the cow was offered water, but she refused to drink. At 7.48 a. m. the cow was lying; during all other readings she was standing. At 9.25 a. m. the reading was taken immediately after the cow had urinated. At 7.48 a. m., 3.10 p. m., 5 p. m., and 5.30 p. m. the read-

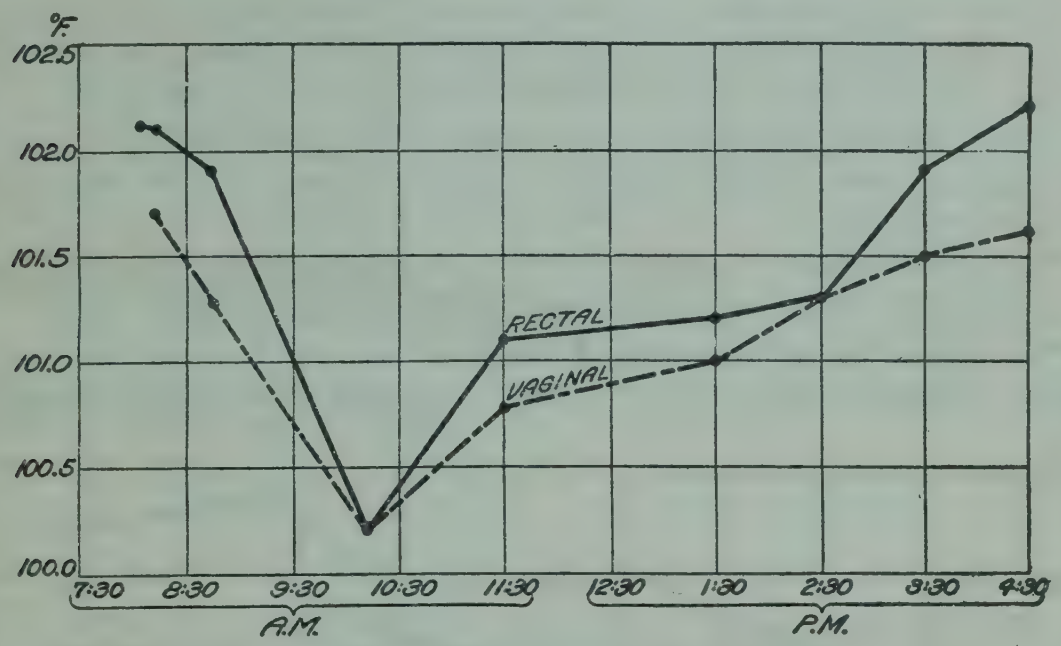

FIG. 6.—Variations in body temperature of cow 885 on December 1, 1919, 8.06 a. m. to 4.30 p. m. From 8.06 a. m. to 4.30 p. m. standing; 8.12 a. m. immediately after defecating; 8.30 a. m. drank 29.4 kgm. of water at 52°F.; 10.12 a. m. immediately after urinating and defecating; 2.30 p. m. after defecating.

ings were taken immediately after defecation. From 5 p. m. to 5.30 p. m. the cow was eating. The results are represented by figure 7.

The striking feature of these curves is that they are very much smoother than the preceding ones. The drop in temperature in the morning is lacking, apparently, because the cow did not drink any water. The curves show instead a very gradual and slight fall in temperature till

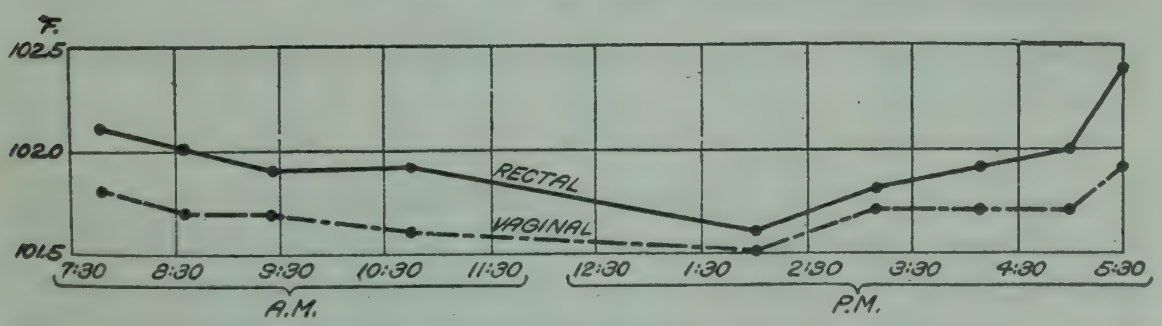

FIG. 7.—Variations in body temperature of cow 885 on December 2, 1919, 7.48 a. m. to 5.30 p. m. At 7.48 a. m. lying and immediately after defecating; 8.36 a. m. to 5.30 p. m. standing; cow drank no water; 9.25 a. m. immediately after urinating; 3.10 p. m. immediately after defecating; 5 p. m. immediately after defecating; 5 p. m. to 5.30 p. m. eating; 5.30 p. m. immediately after defecating.

2 p. m., and from that time a gradual rise. The vaginal curve is lower than the rectal and runs almost parallel with it, not meeting it at any point.

The highest rectal temperature obtained was 102.4° F. at 5.30 p. m., while the lowest was 101.6° at 2 p. m. The highest vaginal temperature obtained was 101.9° at 5.30 p. m., while the lowest was 101.5° at 2 p. m.

OBSERVATIONS ON DECEMBER 3, 1919, WITH COW 885

Readings of the rectal and vaginal temperatures were taken at approximately 1-hour intervals from 8.20 a. m. to 11.12 a. m. and from 1.38 p. m. to 4.48 p. m. The rectal and vaginal temperatures were measured at the same time with the two thermometers interchanged—that is, the one used in figure 7 for the rectum was used here for the vagina, and vice versa. At 8.05 a. m. the cow drank 22 kgm. of water at 55° F. The cow was standing during all observations. At 9 a. m. and 10.12 a. m. the readings were taken immediately after defecation. The results are given in figure 8.

The curves show a marked fall in temperature after the watering. The vaginal is lower than the rectal curve and runs parallel with it.

The highest rectal temperature obtained was 101.9° F. at 2.45 p. m., while the lowest was 100.7° at 9 a. m. The highest vaginal temperature

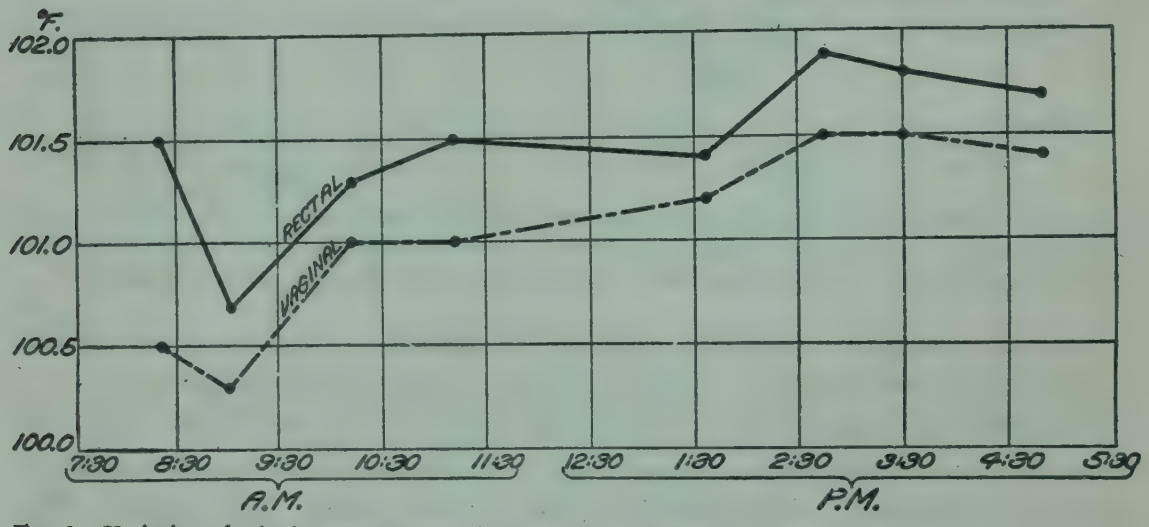

FIG. 8.—Variations in body temperature of cow 885 on December 3, 1919, 8.20 a. m. to 4.48 p. m. At 8.05 a. m. drank 22 kgm. of water at 55° F.; 9 a. m. immediately after defecating; 10.12 a. m. immediately after defecating; cow standing during all observations.

obtained was 101.5° at 2.45 p. m. and 3.30 p. m., while the lowest was 100.3 at 9 a. m.

OBSERVATIONS ON DECEMBER 4, 1919, WITH COW 885

Observations on the rectal and vaginal temperatures were made at approximately 1-hour intervals from 8 a. m. to 11.36 a. m. and from 1.42 p. m. to 5.30 p. m. The rectal and vaginal temperatures were measured at the same time by the two thermometers as on December 3. At about 8 a. m. the cow was offered water, but she refused to drink. At 10.54 a. m. the cow was lying; during all other observations she was standing. At 9.12 a. m. and 4.45 p. m. the readings were taken immediately after defecation. From 5 p. m. to 5.30 p. m. the cow was eating. The results are represented by figure 9.

The curves show irregularity for the first three hours but are much smoother for the rest of the day. Although meeting each other at the very start, the curves soon diverge and the vaginal remains considerably lower than the rectal throughout their course.

The highest rectal temperature obtained was 102.2° F. at 5.30 p. m., while the lowest was 101.6° at 8 a. m. The highest vaginal temperature obtained was 101.8° at 5.30 p. m., while the lowest was 101.2° at 9.12 a. m., 11.36 a. m., and 1.42 p. m.

OBSERVATIONS ON DECEMBER 5, 1919, WITH COW 885

Readings of the rectal and vaginal temperatures were taken at approximately 1-hour intervals from 7.57 a. m. to 11.30 a. m. and from 1.48 p. m. to 5.30 p. m. The rectal and vaginal temperatures were measured at the same time by the two thermometers as on December 2. At 8.10 a. m. the cow drank 23 kgm. of water at 54° F. The cow was standing during all the readings, except at 9.30 a. m. The readings at 7.57 a. m., 8.45 a. m., 10.42 a. m., and 5.30 p. m. were taken immediately after defecation. From 5 p. m. to 5.30 p. m. the cow was eating. The results are represented by figure 10.

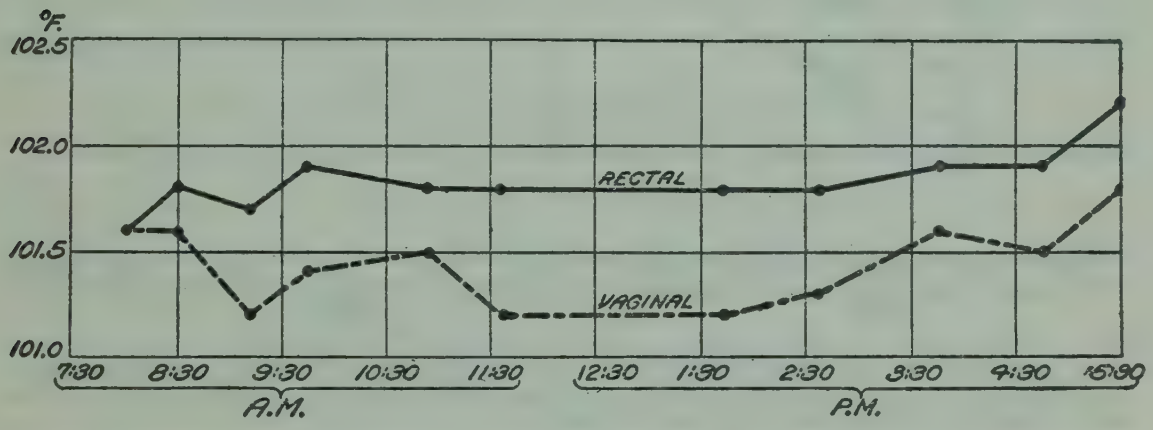

FIG. 9.—Variations in body temperature of cow 885 on December 4, 1919, 8 a. m. to 5.30 p. m. Cow drank no water; 8 a. m. immediately after defecating, standing; 9.12 a. m. immediately after defecating, standing; 10.54 a. m. lying; 11.36 a. m. to 5.30 p. m. standing; 4.45 p. m. immediately after defecating; 5 p. m. to 5.30 p. m. eating.

The curves show a striking resemblance to those in figure 6, showing in about the same way the effect of water drunk, the rise in temperature in the afternoon, as well as the relative positions of the vaginal and rectal curves.

The highest rectal temperature obtained was 102° F. at 5.30 p. m., while the lowest was 100.4° from 8.45 a. m. to 8.56 a. m. The highest vaginal temperature obtained was 101.6° at 5.30 p. m., while the lowest was 99.9° at 8.56 a. m.

GENERAL CONCLUSIONS WITH REGARD TO THE VARIATIONS IN BODY TEMPERATURE FROM ABOUT 7.30 A. M. TO ABOUT 5.30 P. M.

All the foregoing observations indicate that, in cows, the vaginal temperature is decidedly lower than the rectal, when measured at the same depth of 7 inches, while a trend toward parallelism between the two is also apparent. This can be, at least partially, explained by the fact that the vagina is more exposed to the outside atmosphere than is the rectum. For determinations of the average body temperature the

rectum, therefore, is to be preferred to the vagina as a place for inserting the thermometer.

A fall in temperature invariably follows the drinking of water. This fall varies with the quantity of water drunk. Consequently, the first two or three hours after watering do not afford a good time for comparative determinations of body temperature.

After the body has overcome the effect of the water, the temperature appears quite uniform for about three hours, after which there is a gradual

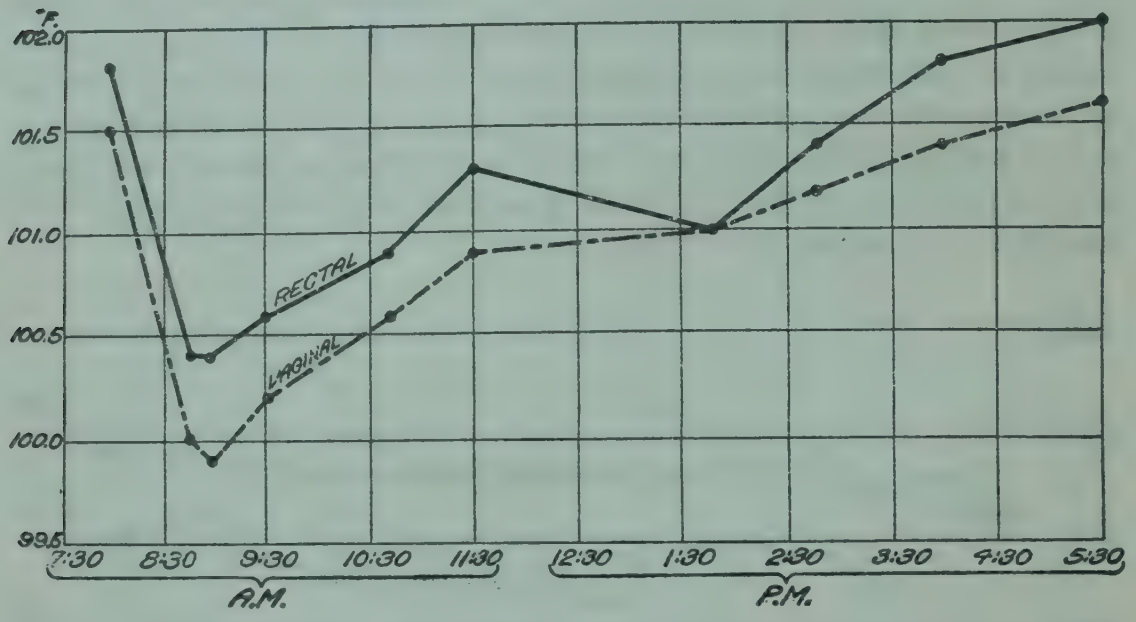

FIG. 10.—Variations in body temperature of cow 885 on December 5, 1919, 7.57 a. m. to 5.30 p. m. At 7.57 a. m. immediately after defecating, standing; 8.10 a. m. drank 23 kgm. at 54° F.; 8.45 a. m. immediately after defecating; 9.30 a. m. lying; 10.42 a. m. immediately after defecating, standing; 5 p. m. to 5.30 p. m. eating; 5.30 p. m. immediately after defecating.

rise which reaches its maximum at about 5.30 p. m. The accelerated rise, however, from 5 p. m. to 5.30 p. m. may be due to the eating.

Since the exact time when the animal changed its position was not recorded during these observations, no definite conclusions can be drawn as to what effect the position of the animal has on the body temperature.

The act of defecation did not produce any noticeable effect.

The highest rectal temperature obtained during this series of observations was 102.4° F. at 5.30 p. m. (fig. 7). The lowest rectal temperature was 100.2° at 10.12 a. m. (fig. 6). The highest vaginal temperature obtained was 102° at 2.57 p. m., 5.20 p. m. (fig. 2), and 5.24 p. m. (fig. 5), while the lowest was 99.9° at 8.56 a. m. (fig. 10).

OBSERVATIONS ON THE THERMAL GRADIENT

Recent investigations on the body temperature of man[1] and other animals[2] show that variations in body temperature depend to a very great extent on the depth to which the thermometer is inserted, and that

[1] BENEDICT, Francis G., and SLACK, Edgar P. A COMPARATIVE STUDY OF TEMPERATURE FLUCTUATIONS IN DIFFERENT PARTS OF THE HUMAN BODY. V, 73 p., illus. Washington, D. C., 1911. (Carnegie Inst. Washington Pub. 155.)

[2] LIPSCHÜTZ, Alexander. ÜBER DIE ABHÄNGIGKEIT DER KÖRPERTEMPERATUR VON DER PUBERTÄTSDRÜSE. *In* Arch. Gesam. Physiol., Bd. 168, Heft 1/4, p. 177–192, 1 fig. 1917.

the thermal gradient rises sharply for the first few centimeters beneath the surface of the skin and soon reaches a point beyond which the body temperature is not materially increased. To reach this point in man[1] and in the guinea pig[2] 6 or 7 cm. were found to be sufficient. The following observations were made with the view of testing out these findings on cows.

OBSERVATIONS ON THERMAL GRADIENT, WITH COW 886 ON DECEMBER 5, 1919, 2.09 P. M. TO 3.18 P. M.

Observations on the rectal and vaginal temperatures were made simultaneously with the two thermometers. The thermometers were first inserted 4 inches deep, then 5 inches, then 7 inches, then 6 inches, and finally again 7 inches. The results are represented by figure 11, and the actual temperature observations, the time, and the insertions are given in Table I.

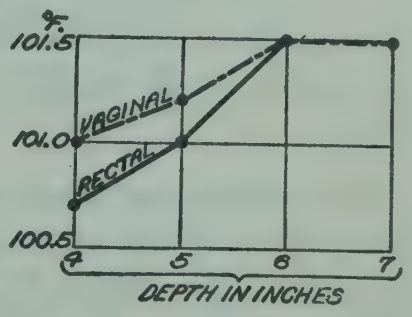

FIG. 11.—Observations on thermal gradient with cow 886 on December 5, 1919, 2.09 p. m. to 3.18 p. m.

TABLE I.—*Thermal gradient observations*

Time.	Rectal temperature.	Vaginal temperature.	Depth inserted.
	° *F.*	° *F.*	*Inches.*
2.09 p. m...	100. 7	101. 0	4
2.12 p. m...	101. 0	101. 2	5
2.15 p. m...	101. 5	101. 5	7
2.24 p. m...	101. 5	101. 5	6
3.18 p. m...	101. 5	.....	7

The results show that at a depth of 4 inches the rectal temperature was 0.80° F. lower than at a depth of either 6 or 7 inches, while it was only 0.3° lower than at a depth of 5 inches. The vaginal temperature was 0.2° lower at a depth of 4 inches than at a depth of 5 inches and 0.5° lower than at a depth of either 6 or 7 inches. There was no rise in temperature between 6 and 7 inches—that is, the maximum body temperature was reached in this case when the thermometer was inserted 6 or 7 inches into the rectum or the vagina. Up to a depth of 6 inches the vaginal curve appears higher than the rectal.

OBSERVATIONS ON THERMAL GRADIENT, WITH COW 886, ON DECEMBER 5, 1919, 3.25 P. M. TO 3.42 P. M.

Readings of the rectal and vaginal temperatures were taken simultaneously as before. The thermometers were first inserted 6 inches deep, then 5 inches, then 4 inches, and finally 7 inches. The results are represented by figure 12, and the actual temperature observations, the time and the insertions are given in Table II.

[1] BENEDICT, Francis G., and SLACK, Edgar P. OP. CIT.
[2] LIPSCHUTZ, Alexander. OP. CIT.

TABLE II.—*Thermal gradient observations*

Time.	Rectal temperature.	Vaginal temperature.	Depth inserted.
	° *F.*	° *F.*	*Inches.*
3.25 p. m...	101. 4	101. 5	6
3.30 p. m...	101. 2	101. 5	5
3.36 p. m...	100. 5	101. 2	4
3.42 p. m...	101. 6	101. 6	7

The results show a very rapid rise between 4 and 5 inches, while beyond that point the rise is much slower. In this case there was a slight rise in temperature from 6 to 7 inches. However, it is very likely that this rise is due not to this difference in depth but to the difference in time, since there was an interval of 17 minutes between the two readings and the tendency of the body temperature is to rise in the afternoon. The vaginal curve is higher than the rectal, but they meet at a depth of 7 inches.

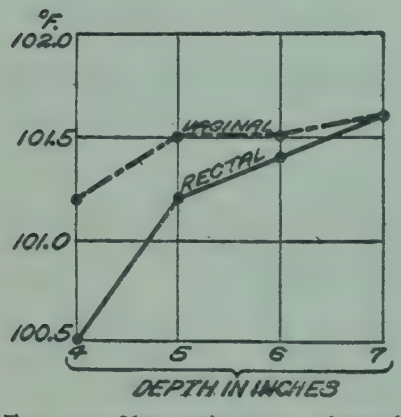

FIG. 12.—Observations on thermal gradient with cow 886 on December 5, 1919, 3.25 p. m. to 3.42 p. m.

OBSERVATIONS ON THERMAL GRADIENT WITH COW 885 ON DECEMBER 5, 1919, 4 P. M. TO 4.18 P. M.

Readings of the rectal and vaginal temperatures were taken simultaneously by the two thermometers as before. The thermometers were inserted first 6 inches deep, then 7 inches, then 4 inches, and finally 5 inches. The results are represented by figure 13, and the actual temperature observations, the time, and the insertions are given in Table III.

TABLE III.—*Thermal gradient observations*

Time.	Rectal temperature.	Vaginal temperature.	Depth inserted.
	° *F.*	° *F.*	*Inches.*
4.00 p. m.....	101. 7	101. 3	6
4.03 p. m.....	101. 8	101. 4	7
4.10 p. m.....	101. 4	101. 0	4
4.18 p. m.....	101. 5	101. 2	5

The results show a very gradual rise from 4 inches to 7 inches, the difference being only 0.4° F. The order in which the readings were taken is significant in showing that despite the tendency of the body temperature to rise at this time of the day, the 4- and 5-inch insertions which followed the 6- and 7-inch insertions show a lower temperature than the latter. The vaginal curve is in this case lower than the rectal and is parallel with it.

OBSERVATIONS ON THERMAL GRADIENT WITH COW 886 ON DECEMBER 5, 1919, 4.24 P. M. TO 4.42 P. M.

Observations on the rectal and vaginal temperatures were made simultaneously by means of the two thermometers as before. The thermometers were first inserted 7 inches deep, then 6 inches, then 5 inches, and finally 4 inches. The results are represented by figure 14, and the actual temperature observations, the time, and the insertions are given in Table IV.

TABLE IV.—*Thermal gradient observations*

Time.	Rectal temperature.	Vaginal temperature.	Depth inserted.
	° *F.*	° *F.*	*Inches.*
4.24 p. m.....	101. 8	101. 6	7
4.30 p. m.....	101. 8	101. 6	6
4.36 p. m.....	101. 7	101. 5	5
4.42 p. m.....	101. 5	101. 2	4
4.48 p. m.....	102. 0	101. 6	7

The results show a more rapid rise in temperature from a depth of 4 to 5 inches than from 5 to 6 inches. There is no change in temperature from a depth of 6 to a depth of 7 inches, thus indicating that the maximum

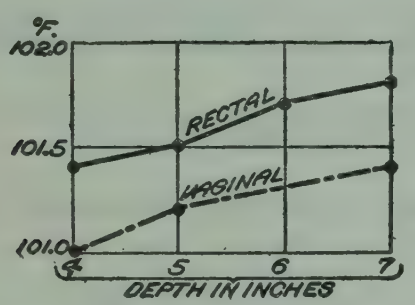

FIG. 13.—Observations on thermal gradient with cow 885 on December 5, 1919, 4 p. m. to 4.18 p. m.

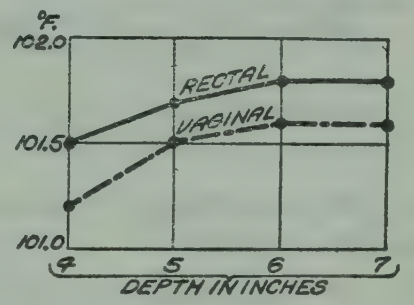

FIG. 14.—Observations on thermal gradient with cow 886 on December 5, 1919, 4.24 p. m. to 4.42 p. m.

temperature was in this case reached at a depth of 6 inches. The reading at 4.48 p. m. is given in the table to show the effect of the time of day. The vaginal curve is lower than the rectal and is parallel with it.

GENERAL CONCLUSIONS WITH REGARD TO THE THERMAL GRADIENT

From a consideration of the results given above it is apparent that the thermal gradient between the temperature at a depth of 4 inches and at a depth of 6 inches is noticeable, while there is no material change between a depth of 6 inches and a depth of 7 inches. It may be concluded, therefore, that at a depth of 7 inches the body temperature is essentially at its maximum. This does not show, of course, that the temperature may not be actually higher in some other parts of the body, where there is a specific metabolic activity, but it does show that it is

much safer in the determinations of the average body temperature of a cow to insert the thermometer to a depth of 7 inches than to a depth of 4 or 5 inches.

The fact that the vaginal curve below 6 or 7 inches is in some cases higher than the rectal (fig. 11, 12) and in some cases lower (fig. 13, 14) illustrates the inconstancy of the temperature when measured at a depth below 6 inches.

VARIATIONS IN BODY TEMPERATURE FROM ABOUT 5 P. M. TO ABOUT 7 P. M.

The purpose of making temperature observations from about 5 p. m. to about 7 p. m. is twofold: First, to study the effect of the feed on the body temperature and, second, to get some idea as to the temperature fluctuations at 6 p. m., the time representing the beginning as well as

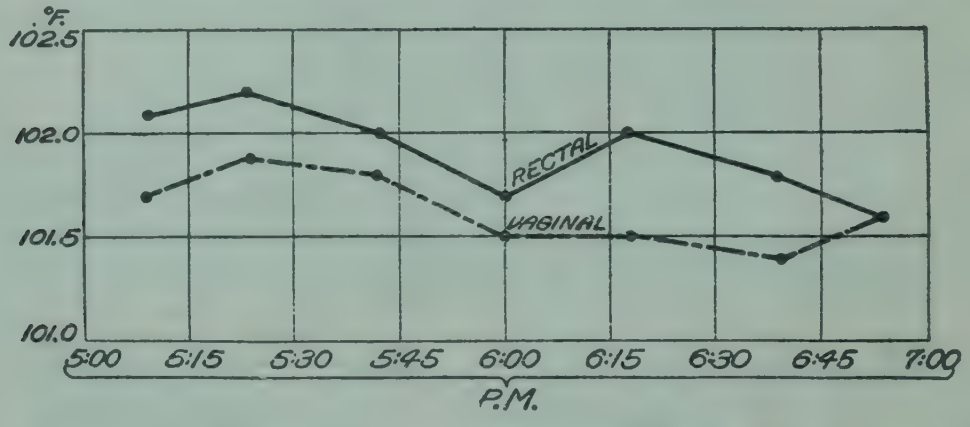

FIG. 13.—Variations in body temperature of cow 886 on December 9, 1919, 5.10 p. m. to 6.54 p. m. From 5 p. m. to 5.42 p. m. eating; 6.18 p. m. to 6.54 p. m. lying.

the end of experimental periods in the respiration calorimeter experiments at the Institute.

OBSERVATIONS ON DECEMBER 9, 1919, WITH COW 886

Observations on the rectal and vaginal temperatures were made at approximately 15-minute intervals from 5.10 p. m. to 6.54 p. m. The cow was eating from 5 p. m. to 5.42 p. m. From 6.18 p. m. to 6.54 p. m. she was lying. The results are represented by figure 15.

The curves show a slight rise in temperature from 5.10 p. m. to 5.25 p. m., followed by a gradual fall till 6 p. m. After 6 p. m. the rectal curve again shows a rise till 6.18 p. m., followed by a gradual drop till 6.54 p. m., when it meets the vaginal curve. The vaginal curve is lower than the rectal and is parallel with it only till 6 p. m.

OBSERVATIONS ON DECEMBER 10, 1919, WITH COW 886

Readings of the rectal and vaginal temperatures were taken at approximately 15-minute intervals from 5 p. m. to 6.30 p. m. From 5 p. m. to 5.36 p. m. the cow was eating. The reading at 5 p. m. was taken immediately after defecation. The cow was lying from 6.03 p. m. to 6.30 p. m. The results are given in figure 16.

The curves show, in general, a slight and gradual drop. It should be noted that the initial temperature—that is, the temperature at 5 p. m.—was rather high.

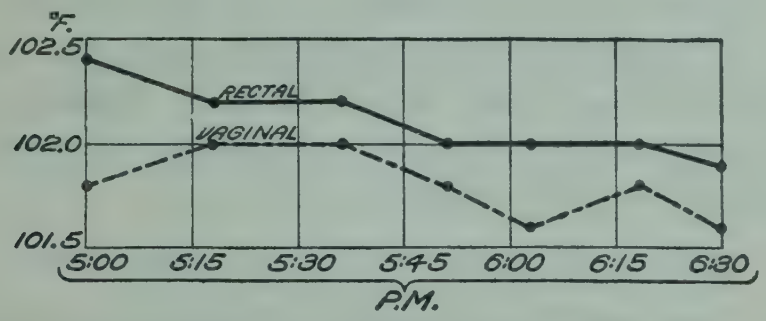

FIG. 16.—Variations in body temperature of cow 886 on December 10, 1919, 5 p. m. to 6.30 p. m. From 5 p. m. to 5.36 p. m. eating; 5 p. m. immediately after defecating; 6.03 p. m. to 6. 30 p. m. lying.

OBSERVATIONS ON DECEMBER 11, 1919, WITH COW 886

Readings of the rectal and vaginal temperatures were taken at approximately 15-minute intervals from 4.52 p. m. to 6.36 p. m. From 5 p. m. to 5.48 p. m. the cow was eating. From 6.21 p. m. to 6.36 p. m. she was lying. The results are represented by figure 17.

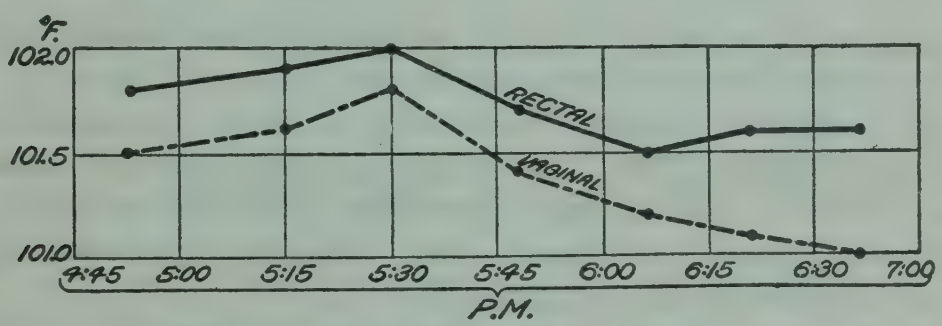

FIG. 17.—Variations in body temperature of cow 886 on December 11, 1919, 4.52 p. m. to 6.36 p. m. From 5 p. m. to 5.48 p. m. eating; 6.21 p. m. to 6.36 p. m. lying.

The curves show a gradual rise in temperature till 5.30 p. m., followed by a fall which is continuous in the vaginal temperature but is interrupted by a very slight rise in the rectal temperature at 6.21 p. m., the time the cow lay down. The two curves are nearly parallel.

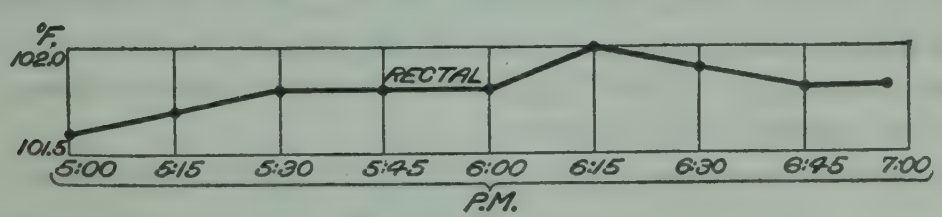

FIG. 18.—Variations in body temperature of cow 886 on December 13, 1919, 5 p. m. to 6.57 p. m. From 5 p. m. to 5.30 p. m. eating; 6.15 p. m. to 6.57 p. m. lying.

OBSERVATIONS ON DECEMBER 13, 1919, WITH COW 886

Observations on the rectal temperature were made at about 15-minute intervals from 5 p. m. to 6.57 p. m. From 5 p. m. to 5.30 p. m. the cow was eating. From 6.15 p. m. to 6. 57 p. m. she was lying. The results are given in figure 18.

The curve shows, in general, a gradual rise till 6.15 p. m., the time the cow lay down, followed by a slight and gradual drop.

OBSERVATIONS ON DECEMBER 14, 1919, WITH COW 886

Observations on the rectal temperature were made at approximately 15-minute intervals from 5.06 p. m. to 6.21 p. m. From 5 p. m. to 5.36 p. m. the cow was eating. From 5.50 p. m. to 6.21 p. m. she was lying. The results are represented by figure 19.

OBSERVATIONS ON JANUARY 12, 1920, WITH COW 886

Readings of the rectal temperature were taken at 15-minute intervals from 5 p. m. to 6 p. m. The cow was eating from 5 p. m. to 6. p. m. The results are given in figure 20.

The curve shows, in general, a gradual rise from 5 p. m. to 6 p. m. It should be mentioned that the cow received a supermaintenance ration at this date and that she spent the whole hour from 5 p. m. to 6 p. m. in eating.

OBSERVATIONS ON DECEMBER 11, 1919, WITH COW 885

Readings of the rectal and vaginal temperatures were taken at approximately 15-minute intervals from 5 p. m. to 6. 30 p. m. From 5 p. m. to 5.42 p. m. the cow was eating. The reading at 5 p. m. was taken immediately after defecation. At 6.15 p. m. the cow defecated and pushed out the thermometer. The readings taken then and also immediately after defecation corresponded with each other. The cow was standing all the time. The results are given in figure 21.

The rectal curve shows a gradual rise in temperature till 5.42 p. m., the time the cow was through eating, followed by a slight fall in temperature till 6.15 p. m., followed again by a rise. The vaginal curve is much lower than the rectal.

OBSERVATIONS ON DECEMBER 13, 1919, WITH COW 885

Readings of the rectal temperature were taken at approximately 15-minute intervals from 5.12 p. m. to 7 p. m. From 5 p. m. to 5.35 p. m. the cow was eating. From 6.35 p. m. to 7 p. m. she was lying. The results are represented by figure 22.

The curve is, in a general way, similar to the rectal curve in figure 21.

OBSERVATIONS ON DECEMBER 14, 1919, WITH COW 885

Observations on the rectal temperature were made at approximately 15-minute intervals from 5.18 p. m. to 6.12 p. m. From 5 p. m. to 5.35 p. m. the cow was eating. The cow was up all the time. The results are given in figure 23.

The peculiar feature of this curve is the rather rapid drop from 5.30 p. m. to 6.12 p. m.

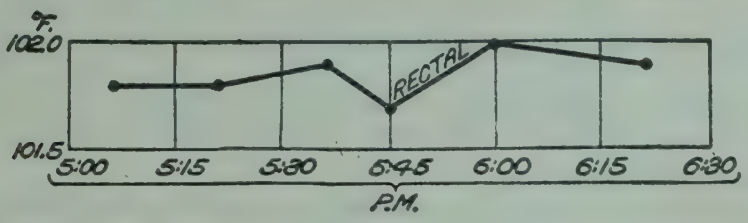

FIG. 19.—Variations in body temperature of cow 886 on December 14, 1919, 5.06 p. m. to 6.21 p.m. From 5 p. m. to 5.36 p. m. eating; 5.50 p. m. to 6.21 p. m. lying.

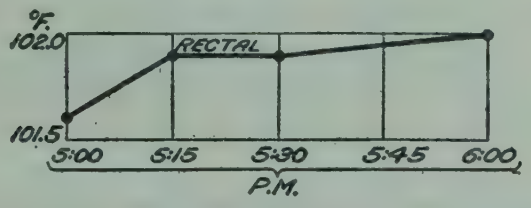

FIG. 20.—Variations in body temperature of cow 886 on January 12, 1920, 5 p. m. to 6 p. m. From 5 p.m. to 6 p. m. eating.

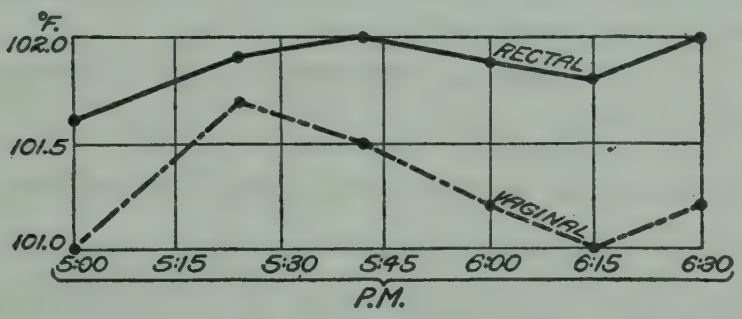

FIG. 21.—Variations in body temperature of cow 885 on December 11, 1919, 5 p. m. to 6.30 p. m. From 5 p. m. to 5.42 p. m. eating; 5 p. m. immediately after defecating; 6.15 p. m. immediately before and after defecating; cow standing all the time.

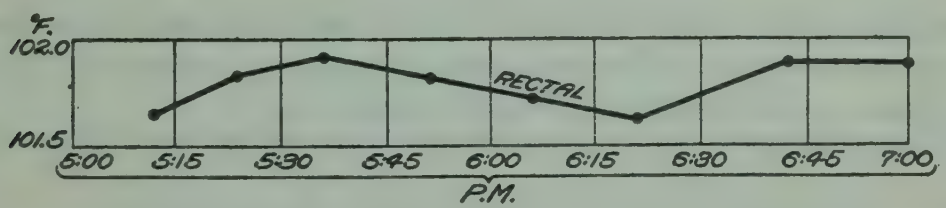

FIG. 22.—Variations in body temperature of cow 885 on December 13, 1919, 5.12 to 7 p. m. From 5 p. m. to 5.35 p. m. eating; 6.35 p. m. to 7 p. m. lying.

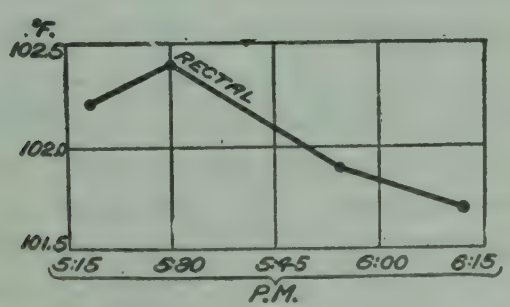

FIG. 23.—Variations in body temperature of cow 885 on December 14, 1919, 5.18 p. m. to 6.12 p. m. From 5 p. m. to 5.35 p. m. eating; cow standing all the time.

OBSERVATIONS ON JANUARY 4, 1920, WITH COW 885

Observations on the rectal temperature were made at approximately 15-minute intervals from 5 p. m. to 5.55 p. m. From 5 p. m. to 5.36 p. m. the cow was eating. The cow was up all the time. The results are represented by figure 24.

OBSERVATIONS ON JANUARY 4, 1920, WITH COW 886

Readings of the rectal temperature were made at 15-minute intervals from 5 p. m. to 6 p. m. The cow was not fed till 6 p. m. and was standing all the time. The results are represented by figure 25.

The peculiar feature of the curve is the fall in temperature till 5.30 p. m., followed by a rise from 5.30 p. m. to 6 p. m.

OBSERVATIONS ON MARCH 20, 1920, WITH COW 886

Two readings of the rectal temperature were taken between 5 p. m. to 6.25 p. m. The cow was not fed till 6.25 p. m. and was standing all the time. The results are given in figure 26.

The curve does not show any material change in temperature from 5.15 p. m. to 6.25 p. m.

OBSERVATIONS ON JANUARY 12, 1920, WITH COW 885

Readings of the rectal temperature were taken at approximately 20-minute intervals from 5 p. m. to 6.04 p. m. The cow was not fed till 6 p. m. and was standing all the time. The results are given in figure 27.

OBSERVATIONS ON FEBRUARY 16, 1920, WITH COW 885

Readings of the rectal temperature were taken at half-hour intervals from 5 p. m. to 6 p. m. The cow was not fed till 6 p. m. and was standing all the time. The results are given in figure 28.

The curve shows a fall in temperature from 5 p. m. to 5.30 p. m.

GENERAL CONCLUSIONS WITH REGARD TO THE VARIATIONS IN BODY TEMPERATURE FROM ABOUT 5 P. M. TO ABOUT 7 P. M.

A comparison of the temperature curves obtained when the cow was fed at 5 p. m. with those obtained when the cow was not fed till 6 p. m. shows, in general, that when the cow was fed at 5 p. m. there was a slight rise in temperature till about 5.30 p. m., followed by a slight fall. This rise from 5 p. m. till 5.30 p. m. has been noted in previous observations (fig. 2, 5, 7, 9, 10), when the cows were fed at 5 p. m. When, however, the cow was not fed till 6 p. m., the temperature in most cases not only did not rise from 5 p. m. to 5.30 p. m., but instead dropped slightly. This indicates that eating of the feed raises the body temperature slightly for about ½ hour when the cows receive a maintenance ration.

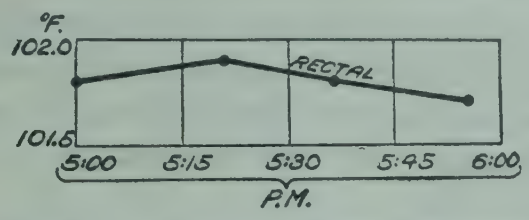

FIG. 24.—Variationsi n body temperature of cow 885 on January 4, 1920, 5 p. m. to 5.55 p. m. From 5 p. m. to 5.36 p. m. eating; cow standing all the time.

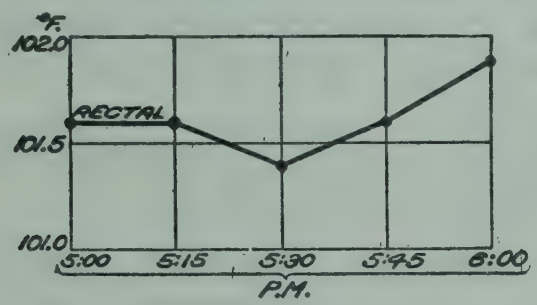

FIG. 25.—Variations in body temperature of cow 886 on January 4, 1920, 5 p. m. to 6 p. m. Cow not fed till 6 p. m.; standing all the time.

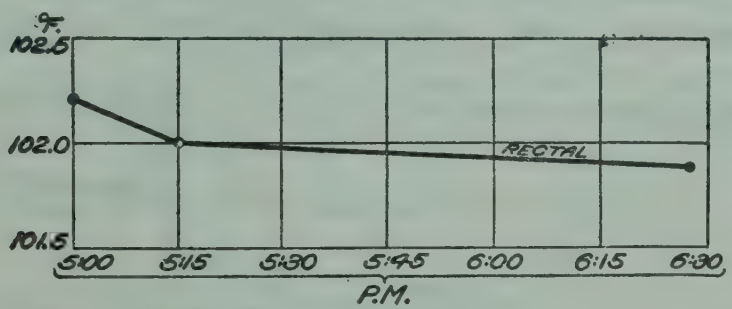

FIG. 26.—Variations in body temperature of cow 886 on March 20, 1920, 5 p. m. to 6.25 p. m. Cow not fed till 6.25 p. m.; standing all the time.

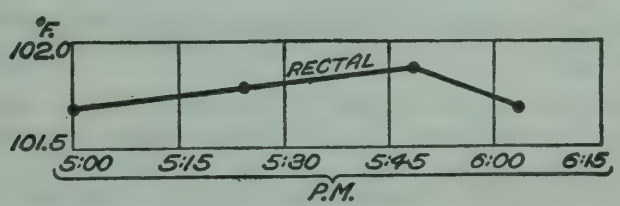

FIG. 27.—Variations in body temperature of cow 885 on January 12, 1920, 5 p. m. to 6.04 p. m. Cow not fed till 6 p. m.; standing all the time.

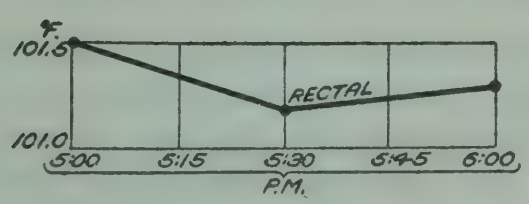

FIG. 28.—Variations in body temperature of cow 885 on February 16, 1920, 5 p. m. to 6 p. m. Cow not fed till 6 p. m.; standing all the time.

The subsequent fall in temperature can be ascribed partly to the fact that the temperature of the feed is lower than that of the body, thus tending to lower somewhat the temperature of the latter, and partly to the natural tendency of the body temperature to drop after a stimulated rise.

The act of defecation did not produce any effect.

The highest rectal temperature obtained during these observations was 102.4° F. (fig. 16), while the lowest was 101.2° (fig. 28). The highest vaginal temperature obtained was 102°, while the lowest was 101.°

The rectal temperature at 6 p. m. fluctuates in all cases, except in one (fig. 28), between 101.5° and 102° F.

OBSERVATIONS ON THE EFFECT OF CHANGE IN POSITION ON BODY TEMPERATURE

It is an established fact that an animal produces more heat when standing than when lying. This being the case, one might, therefore,

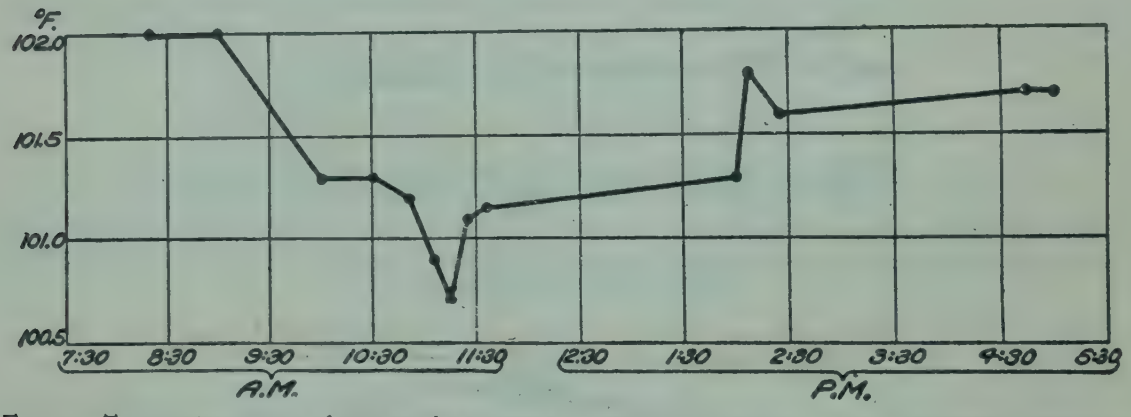

FIG. 29.—Temperature curve of cow 886 for January 5, 1920, 8.20 p. m. to 5 p. m., including effects of change in position. At 8.20 a. m. lying; 8.40 a. m. drank 13.6 kgm. of water at 50° F.; 9 a. m. just lay down; 9 a. m. to 10.30 a. m. lying; 10.31 a. m. forced to get up; 10.50 a. m. immediately before defecating; 10.54 a. m. immediately after defecating; 11.06 a. m. lying since 10.57 a. m.; 11.15 a. m. still lying; 11.24 a. m. up since 11.20 a. m.; 11.36 a. m. still up; 2 p. m. just lay down; 2.02 p. m. forced to get up; 2.06 p. m. up since 2.02 p. m.; 2.24 p. m. down since 2.20 p. m.; 4.45 p. m. down since 4 p. m.; 4.48 p. m. forced to get up; 5 p. m. immediately after defecating.

expect some changes in the temperature of the body when the animal changes its position. Although the position of the animal was noted in the previous observations, the changes were not frequent enough to show any effect. The following observations were made chiefly with the view of studying the possible effects on the body temperature that may arise from a forced or voluntary change in position.

Figure 29 represents observations made on the rectal temperature of cow 886 on January 5, 1920, from 8.20 a. m. to 5 p. m.

The most striking features of the curve are the sudden fluctuations between 10.50 a. m. and 11.24 a. m., and between 2 p. m. and 2.20 p. m. The accelerated fall in temperature (effect of the water) from 10.57 a. m.

to 11.15 a. m. in the former case appear to be due largely to a change from a standing to a lying position, while the subsequent sudden rise at 11.24 a. m. is apparently due largely to the change from a lying to a

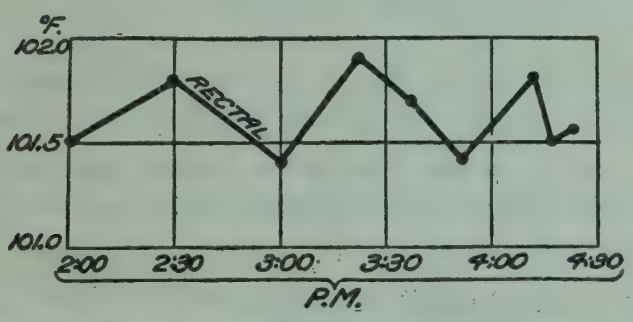

FIG. 30.—Temperature curve of cow 886 for January 13, 1920, 2 p. m. to 4.22 p. m., including effects of change in position. From 2 p. m. to 3.20 p. m. forced to get up; 3.22 p. m. immediately after defecating, urinating with thermometer in rectum; 3.52 p. m. lying since 3.48 p. m.; 4.16 p. m. just got up; 4.22 p. m. still up.

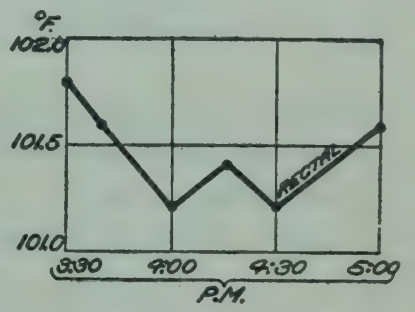

FIG. 31.—Temperature curve of cow 886 for January 15, 1920, 3.30 p. m. to 5 p. m., including effects of change in position. At 3.30 p. m. lying; 3.32 p. m. forced to get up; 3.40 p. m. to 5 p. m. standing; 4.15 p. m. urinated with thermometer in rectum.

standing position. In the second case the sudden rise from 2 p. m. to 2.06 p. m. appears to be due to the change from a lying to a standing position, while the subsequent drop at 2.24 p. m. apparently represents

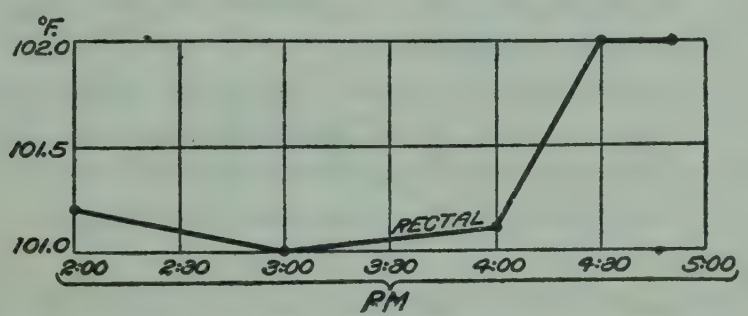

FIG. 32.—Temperature curve of cow 886 on January 30, 1920, 2 p. m. to 4.50 p. m., including effects of change in position. At 2 p. m. lying; 2.43 p. m. to 4.50 p. m. standing.

the effect of the change from the standing to a lying position and also perhaps the tendency for the body temperature to come back to normal. It should be noted that in both cases the effect is not lasting.

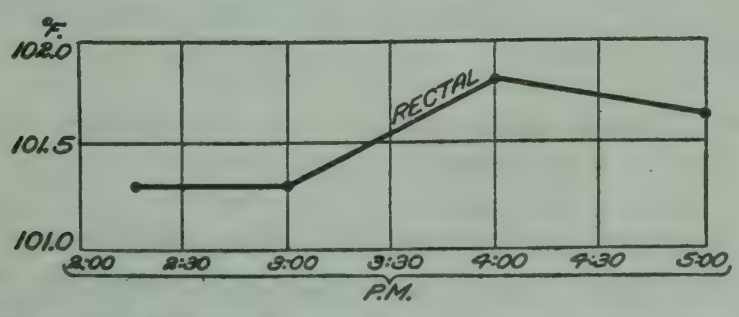

FIG. 33.—Temperature curve of cow 886 on February 16, 1920, 2.15 p. m. to 5 p. m., including effects of position. From 2.15 p. m. to 5 p. m. standing.

A careful study of figures 30 to 35 reveals that, in general, the position of the animal hardly affects the normal course of the fluctuations of its body temperature, for, while the fluctuations were considerable

and rather irregular at the time when the cow did not change her position (fig. 31, 32, 35), the temperature remained fairly uniform when the position was changed (fig. 32 and especially fig. 34).

DAILY VARIATIONS IN BODY TEMPERATURE MEASURED AT 5 P. M.

Inasmuch as at the Institute of Animal Nutrition the determinations of the heat produced by cows by means of the respiration calorimeter are made during a period of 24 or 48 hours, it appears that a knowledge of the daily variations in the body temperature at exactly the same time of the day, corresponding to the beginning and the end of the period, is most essential, as far as those experiments are concerned. That the body temperature of the same animal varies from day to day and from hour to hour has been shown by most of the foregoing observations, even by those made during the time when there was no influence of water or feed (fig. 25–28). To study the extent of the daily fluctuations in body temperature determined at exactly the same time of the day was the object of the next series of observations. To avoid any effect of water drunk or of feed, the observations were made just before feeding at exactly 5 p. m.

Figures 36 to 38 represent the temperatures of cow 886 measured at 5 p. m. for the days there indicated.

All these curves resemble each other very much, showing considerable fluctuations. In figure 36 the lowest temperature is 101.6° F., while the highest is 102.4°. In figure 37 the lowest temperature is 101.4°, while the highest is 102°. In figure 38 the lowest temperature is 101.7°, while the highest is 102.2°.

Figures 39 to 41 represent the temperatures of cow 885 measured at 5 p. m. for the days there indicated.

These three curves resemble each other very much, but they are different from the three preceding curves of cow 886 in that they show much less variation. The lowest temperature in figure 39 is 101.6° F., while the highest is 101.9°. In figure 40 the lowest temperature is 101.8°, while the highest is 101.9°. In figure 41 the lowest temperature is 101.5°, while the highest is 101.8°.

GENERAL CONCLUSIONS WITH REGARD TO THE DAILY VARIATIONS IN TEMPERATURE AT 5 P. M.

The foregoing observations show that daily fluctuations in body temperature depend to a large extent on the individuality of the cow. It is, therefore, not safe to assume in every case that the body temperature of a cow is the same at the same hour of the day, even under normal conditions.

Variations of 0.5° or 0.6° F. in the body temperature of the same animal (886) measured at exactly the same time of the day for several

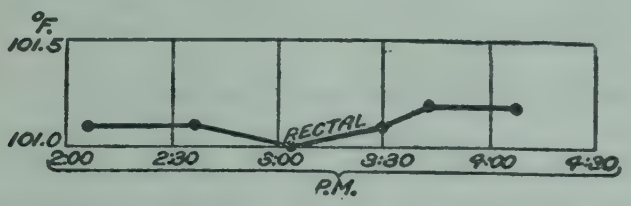

FIG. 34.—Temperature curve of cow 885 for January 13, 1920, 2.06 p. m. to 4.06 p. m., including effects of change in position. At 2.06 p. m. standing; 2.36 p. m. standing; 3.04 p. m. down since 2.48 p. m.; 3.30 p. m. still down; 3.42 p. m. up since 3.38 p. m.; 4.06 p. m. still up.

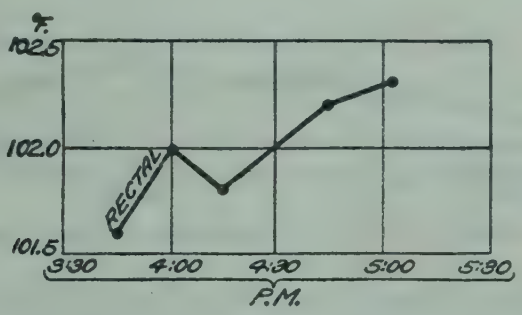

FIG. 35.—Temperature curve of cow 885 for March 22, 1920, 3.45 p. m. to 5.03 p. m., including effects of position. From 3.45 p. m. to 5.03 p. m. standing.

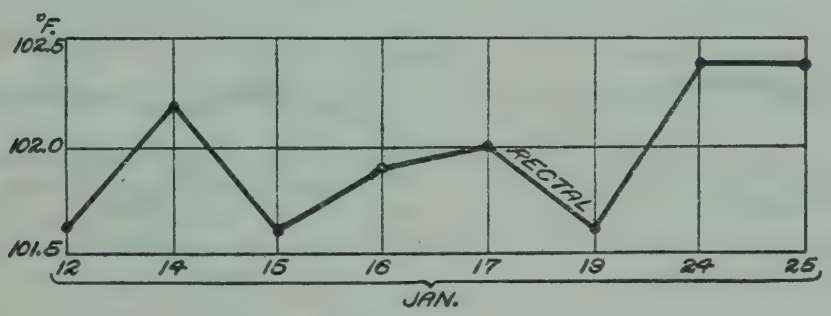

FIG. 36.—Temperature curve of cow 886 for January 12 to January 25, 1920, 5 p. m.

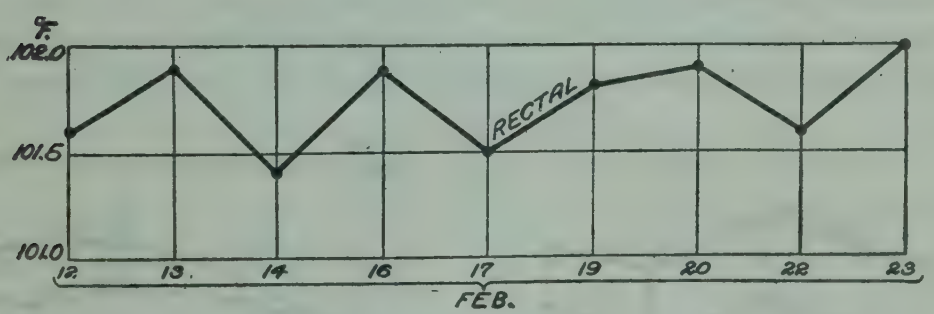

FIG. 37.—Temperature curve of cow 886 for February 12 to February 23, 1920, 5 p. m.

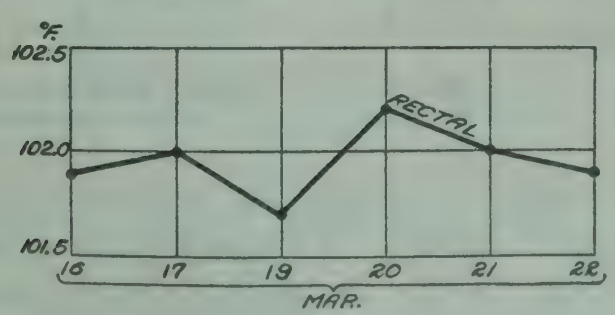

FIG. 38.—Temperature curve of cow 886 for March 16 to March 22, 1920, 5 p. m.

consecutive days are quite common, and greater ones are possible even under identical conditions.

DAILY VARIATIONS IN BODY TEMPERATURE MEASURED AT 8 A. M., 1.30 P. M., AND AT 9 A. M., RESPECTIVELY, FOR SEVERAL CONSECUTIVE DAYS

It has been shown by the very first observations (fig. 1–10) what effect the water drunk under the conditions of the experiment has on

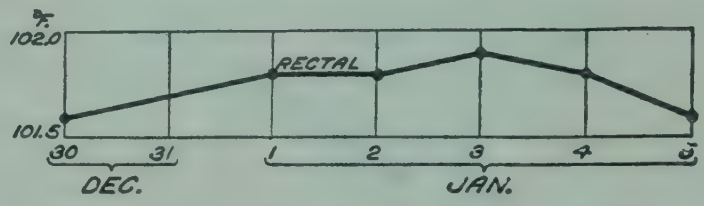

FIG. 39.—Temperature curve of cow 885 for December 30, 1919, to January 5, 1920, 5 p. m.

the body temperature—that if water is drunk in considerable quantity the fall in temperature is very marked, and this effect may last more than three hours, but that after this effect is overcome the temperature

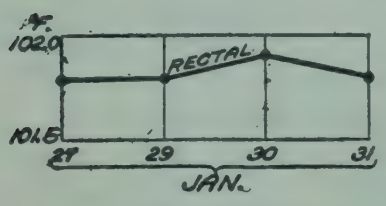

FIG. 40.—Temperature curve of cow 885 for January 27 to January 31, 1920, 5 p. m.

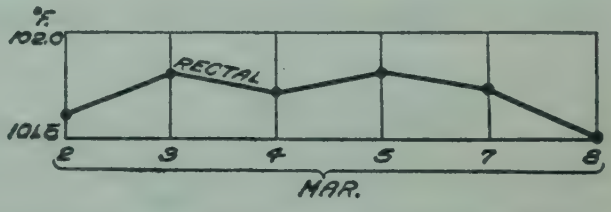

FIG. 41.—Temperature curve of cow 885 for March 2 to March 8, 1920, 5 p. m.

remains quite uniform for about the next three hours. It would therefore perhaps be interesting to see a graphic representation of the temperature changes for several days when measured, first, before watering,

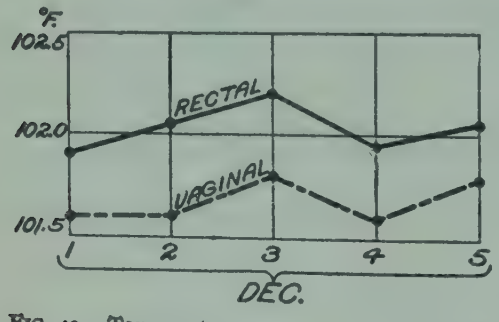

FIG. 42.—Temperature curve of cow 886 for December 1 to December 5, 1919, 8 a. m., before watering.

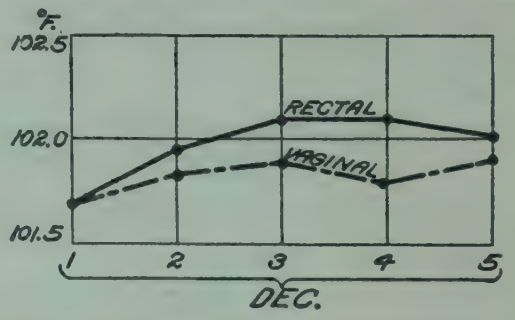

FIG. 43.—Temperature curve of cow 886 for December 1 to December 5, 1919, 1.30 p. m.

second, when the effect of the water has been overcome, and, third, about an hour after watering. Accordingly, the respective temperatures observed during the first few days of the experiment (fig. 1–10), at 8 a. m., at 1.30 p. m., and at 9 a. m., are shown by figures 42 to 46.

Figures 42 and 43 represent the temperatures of cow 886 at 8 a. m. and at 1.30 p. m., respectively, for December 1 to December 5, 1919.

Figures 44 and 45 represent the temperatures of cow 885 at 8 a. m. and at 1.30 p. m., respectively, for December 1 to December 5, 1919. In figure 44 the temperature for December 3 is omitted because the observations did not begin till 8.20 a. m. that day.

On comparing these curves (fig. 42–45) with those obtained at 5 p. m. for the two cows (fig. 36–41) it is observed that while the temperature

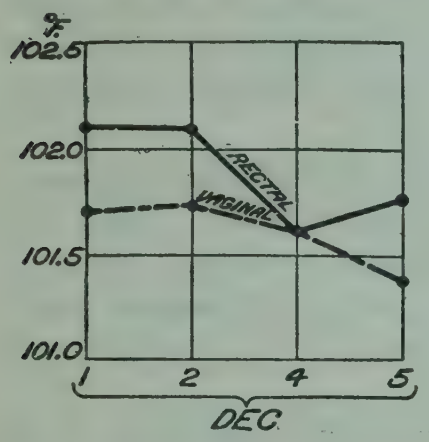

FIG. 44.—Temperature curve of cow 885 for December 1 to December 5, 1919, 8 a. m., before watering.

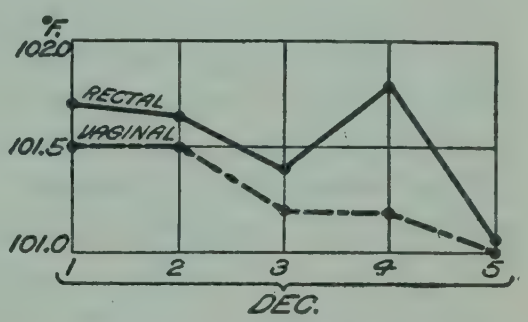

FIG. 45.—Temperature curve of cow 885 for December 1 to December 5, 1919, 1.30 p. m.

of cow 886 at 5 p. m. fluctuated to a large extent from day to day, her body temperature at 8 a. m. or at 1.30 p. m. varied relatively little. On the other hand, the temperature of cow 885, which is very uniform at 5 p. m., varies considerably more at 8 a. m. or at 1.30 p. m. These differ-

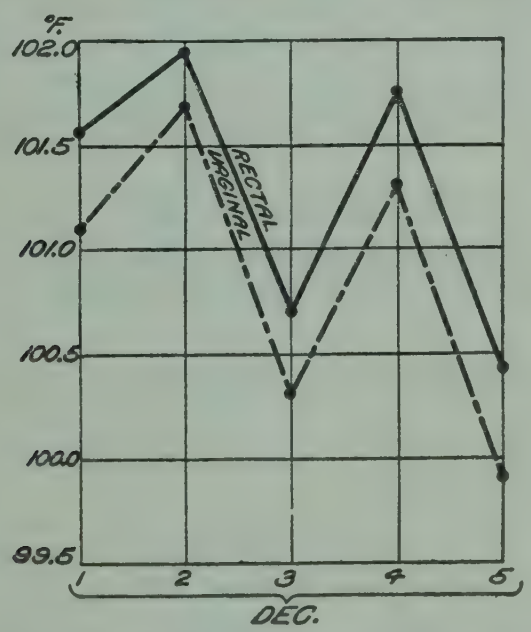

FIG. 46.—Temperature curve of cow 885 for December 1 to December 5, 1919, 9 a. m., showing the effect of water. On December 1 cow drank 29.4 kgm. at 8.30 a. m. On December 2 she refused to drink. On December 3 she drank 22 kgm. at 8.05 a. m. On December 4 she refused to drink. On December 5 she drank 23 kgm. at 8.10 a. m.

ences, apparently due to the individuality of the cow, can not be explained.

Figure 46, representing the temperatures of cow 885 at 9 a. m. for December 1 to December 5, 1919, shows very strikingly the possible

differences in body temperature when measured at exactly the same time of the day but under the influence of the water drunk.

SUMMARY OF RESULTS

The several series of temperature observations on two dry cows lead to the following conclusions:

(1) The rectal temperature is higher than the vaginal when measured at the same depth of 7 inches, showing an average excess of about 0.3° F. The relative values, however, vary under different conditions but show a trend toward parallelism.

(2) A fall in temperature invariably follows the drinking of water. This fall varies directly with the quantity of water drunk. After the effect of the water drunk in the morning has been overcome, the temperature remains fairly constant till about 2.30 p. m. When no water is drunk, the temperature is practically constant in the morning and in the afternoon till about 2.30 p. m.

(3) There is a gradual rise in temperature in the afternoon from about 2.30 p. m. to about 5 p. m.

(4) Eating of feed raises the body temperature slightly for about ½ hour when the cows receive a maintenance ration.

(5) The temperature of the rectum or vagina is decidedly higher when measured as a depth of 6 or 7 inches than at a depth of 4 or 5 inches, thus indicating a temperature gradient.

(6) There is no material change in temperature between a depth of 6 inches and a depth of 7 inches, whereas there is a distinct difference in temperature between a depth of 4 inches and a depth of 6 inches, thus showing the unreliability of measuring the temperature at a depth of less than 6 inches.

(7) The position of the animal has hardly any effect on the body temperature, but there is some indication that the temperature is slightly affected when measured shortly after the change in position has been made (fig. 29).

(8) There is no difference in temperature when measured before or after defecation.

(9) Daily fluctuations in body temperature depend to a great extent on the individuality of the cow.

(10) A variation of 0.8° F. in the rectal temperature of the same animal was observed, when measured at the same time of the day under identical conditions and outside the influence of water or feed (fig. 36), while under the influence of water a difference of 1.3° was observed in two consecutive days measured at the same time of the day (fig. 46).

EFFECT OF TIME OF IRRIGATION ON KERNEL DEVELOPMENT OF BARLEY

By HARRY V. HARLAN, *Agronomist in Charge of Barley Investigations*, and STEPHEN ANTHONY, *formerly Assistant, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*

The studies of kernel development of barley made at Aberdeen, Idaho,[1] in 1915 and 1916, and previously reported,[2] gave such satisfactory results that in 1917 an attempt was made to use this method of inquiry in a study of the effect of irrigation water. It is the common practice in irrigated districts so to arrange the applications of water that the last irrigation is given a considerable time before maturity, usually shortly after flowering. This, of course, is to prevent lodging, for as the weight of the grain increases, the chances of lodging are increased. There is also a widespread belief that during the later stages of ripening little absorption from the soil solution takes place and that the final maturation of the kernel can take place in the shock as well as before cutting. This latter belief was not substantiated in the work of 1915 and 1916, but there seemed to be no definite information as to how late in its growth period the plant could use water or just what occurred when a plant suffered from lack of water.

Although it was hoped that some information could be gained on the general response to applications of water, the effect of delayed irrigation was made the primary object of the experiment. The factors affected by irrigation are so numerous and so involved that the final statement of yield per acre does not afford much basis for interpretation. While the method used may not show any direct relation to yield, it does show the effect on kernel development. The development of the individual kernels is one of the three factors of yield, the other two being the number of kernels per spike and the number of spikes per unit area. The period during which the quantity of water or the application of water affects the size of the kernel is worth determining. Knowledge of the period during which the application of water affects the growth or maturation of the plant affords a basis for the better understanding of irrigation.

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[1] These studies were made on the Aberdeen Substation, Aberdeen, Idaho, in connection with cereal experiments conducted cooperatively by the Idaho Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

[2] HARLAN, Harry V. DAILY DEVELOPMENT OF KERNELS OF HANNCHEN BARLEY FROM FLOWERING TO MATURITY AT ABERDEEN, IDAHO. *In* Jour. Agr. Research, v. 19, no. 9, p. 393–430, 17 fig., pl. 83–91. Literature cited, p. 429. 1920.

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PLAN OF THE EXPERIMENT

About $\frac{1}{10}$ acre was seeded to Hannchen barley on the Aberdeen Substation, Aberdeen, Idaho. This area was treated as a unit during the early period of growth. It received its last uniform irrigation on June 23. The plot was then subdivided into 16 smaller plots of uniform size. These were inclosed by checks, and each in turn was surrounded by a drainage ditch. Eight of the 16 plots were chosen for the experiment. Plot 1 was handled so as to secure a normal development. It was irrigated on July 10 and July 29. Plot 8 received no water after the uniform irrigation of June 23. Plots 2 to 7, inclusive, received only one application of water after the irrigation of June 23. Water was applied to plot 2 on July 14, to plot 3 on July 17, to plot 4 on July 20, to plot 5 on July 23, to plot 6 on July 26, and to plot 7 on July 29. After the irrigation of July 10, all water was measured, each plot receiving 300 gallons on the dates mentioned, except plot 2, which received only 290 gallons.

Samples were taken on plots 1, 2, and 8 daily from flowering to maturity. Plots 3 to 7, inclusive, were considered to be identical with plot 8 until irrigated. Samples were taken on the day of irrigation either before or soon after the application of water, and daily from that time until maturity. The methods of sampling and of obtaining and recording data have been described previously, and the description will not be repeated here. The results are more than comparable with those reported in the paper on the daily development of the kernels. They are a part of the same data. The data from plot 1 formed the basis of the previous paper. As the data showing daily development have been reported in full for plot 1, all daily data will be omitted here. The summaries in the tables were obtained in the same manner as the summaries previously reported. Slight discrepancies sometimes occur through repeated averages, which do not carry the fraction to sufficient decimal places, and through the fact that a few abnormal kernels are excluded in some cases.

RESULTS

The experimental data recorded are summarized in Table I. The variables recorded were length, lateral diameter, dorsoventral diameter, wet weight, dry weight, water, ash, and nitrogen. All these variables were affected by the time of application of water. The differences are more apparent when considered by variants than when the plot is used as the basis of discussion.

TABLE I.—*Average length, lateral diameter, dorsoventral diameter, and percentage and weight per kernel of dry matter, water, nitrogen, and ash in kernels of Hannchen barley sampled at 24-hour intervals from eight plots variously irrigated at the Aberdeen Substation, Aberdeen, Idaho, in 1917*

PLOT 1

Date.	Length.	Lateral diameter.	Dorsoventral diameter.	Percentage of weight per kernel.				Weight per kernel.				
				Dry matter.	Water.	Nitrogen.[a]	Ash.[a]	Wet weight.	Dry matter.	Water.	Nitrogen.	Ash.
	Mm.	*Mm.*	*Mm.*					*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
July 15.....	2.27	1.26	.....	15.9	84.1	3.75	.....	1.9	0.3	1.6	0.01	.....
16.....	2.88	1.32	0.74	16.0	84.0	4.32	7.91	3.0	.5	2.5	.02	0.05
17.....	3.93	1.39	.82	19.1	80.9	3.06	7.41	4.5	.9	3.6	.03	.06
18.....	6.05	1.57	.90	20.1	79.9	.....	6.14	8.0	1.6	6.4	.....	.09
19.....	7.91	1.79	1.16	22.0	78.0	.....	4.11	12.7	2.8	9.9	.....	.12
20.....	8.78	1.94	1.30	22.4	77.6	2.27	4.22	15.8	3.5	12.3	.08	.15
21.....	9.32	2.10	1.48	24.1	75.9	3.14	3.54	19.2	4.6	14.5	.13	.14
22.....	9.81	2.51	1.71	25.8	74.2	2.33	3.50	25.7	6.6	19.1	.15	.23
23.....	9.96	2.80	1.90	28.5	71.5	2.07	3.64	31.0	8.8	22.2	.18	.32
24.....	9.83	2.86	1.90	30.9	69.1	2.13	2.92	32.7	10.1	22.6	.22	.30
25.....	9.80	3.02	2.05	33.6	66.4	1.94	2.91	36.0	12.1	24.0	.23	.35
26.....	9.77	3.09	2.06	36.2	63.8	1.96	2.67	38.2	13.9	24.4	.27	.37
27.....	9.98	3.30	2.25	37.4	62.6	2.03	2.73	44.6	16.6	28.0	.34	.45
28.....	10.02	3.47	2.33	39.5	60.5	1.98	2.42	48.2	19.0	29.1	.37	.46
29.....	9.84	3.50	2.42	41.2	58.8	2.03	2.46	49.0	20.2	28.8	.41	.50
30.....	9.99	3.59	2.50	43.6	56.4	2.01	2.19	53.0	22.8	30.2	.46	.50
31.....	8.83	3.56	2.54	45.5	54.5	2.12	2.32	52.2	23.7	28.5	.50	.55
Aug. 1.....	9.10	3.62	2.51	48.1	51.9	1.99	2.26	54.8	26.3	28.4	.52	.59
2.....	8.87	3.59	2.54	48.7	51.3	1.90	2.03	53.3	25.9	27.4	.49	.52
3.....	8.87	3.61	2.59	50.3	49.7	1.95	2.05	55.8	28.1	27.8	.55	.58
4.....	8.96	3.60	2.64	50.8	49.2	2.02	1.97	56.3	28.6	27.7	.58	.56
5.....	8.66	3.60	2.62	52.9	47.1	1.89	1.94	55.9	29.5	26.4	.56	.57
6.....	8.78	3.57	2.67	55.1	44.9	2.00	1.89	56.6	31.1	25.5	.62	.59
7.....	8.77	3.60	2.68	56.4	43.6	2.27	1.93	58.0	32.6	25.4	.75	.63
8.....	8.53	3.53	2.64	57.2	42.8	2.28	1.88	56.1	32.0	24.1	.76	.60

PLOT 2

July 16.....	3.2	1.3	0.8	18.1	81.9	3.15	.....	3.4	0.6	2.8	0.02	.....
17.....	3.4	1.4	.9	18.9	81.1	2.63	4.88	4.3	.8	3.5	.02	0.05
18.....	5.6	1.5	.9	19.9	80.1	2.43	6.33	7.0	1.4	5.6	.03	.09
19.....	6.6	1.7	1.0	21.3	78.7	2.11	5.62	8.8	1.9	6.9	.04	.12
20.....	7.9	1.8	1.2	22.1	77.9	2.26	4.91	13.0	2.9	10.1	.07	.13
21.....	9.0	2.0	1.4	23.5	76.5	2.17	.....	17.5	4.1	13.4	.09	.....
22.....	9.6	2.4	1.7	25.6	74.4	2.38	3.48	24.1	6.2	17.9	.11	.27
23.....	9.4	2.6	1.8	27.8	72.2	4.33	3.79	26.9	7.5	19.4	.35	.26
24.....	9.7	2.8	1.9	29.8	70.2	2.28	.....	31.5	9.4	22.1	.21	.....
25.....	9.5	2.8	1.9	32.4	67.6	2.14	.....	31.1	10.1	21.0	.22	.....
26.....	9.9	3.0	2.0	34.0	66.0	1.97	.....	36.3	12.3	24.0	.24	.....
27.....	9.7	3.3	2.1	36.7	63.3	2.16	.....	40.6	14.9	25.7	.32	.....
28.....	9.8	3.3	2.2	36.3	63.7	2.32	.....	42.5	15.4	27.1	.36	.....
29.....	9.7	3.3	2.3	40.4	59.6	2.27	.....	42.5	17.1	25.4	.39	.....
30.....	9.8	3.5	2.3	42.3	57.7	2.02	.....	46.7	19.7	27.0	.40	.....
31.....	8.6	3.4	2.4	45.8	54.2	2.11	.....	48.3	22.2	26.1	.46	.....
Aug. 1.....	8.8	3.4	2.4	45.9	54.1	2.16	.....	47.7	21.9	25.8	.47	.....
2.....	8.7	3.5	2.5	47.2	52.8	2.22	.....	51.9	24.5	27.4	.54	.....
3.....	8.7	3.4	2.3	50.2	49.8	2.41	.....	45.9	23.0	22.9	.55	.....
4.....	8.5	3.5	2.4	50.7	49.3	2.56	.....	47.5	23.8	23.7	.61	.....
5.....	8.7	3.5	2.6	51.5	48.5	2.30	.....	52.2	26.9	25.3	.62	.....
6.....	8.3	3.2	2.3	57.0	43.0	2.39	.....	44.3	25.2	19.1	.60	.....
7.....	8.3	3.3	2.3	59.9	40.1	2.36	.....	45.4	26.6	18.8	.63	.....
8	8.6	3.4	2.5	60.2	39.8	2.54	.....	49.7	29.8	19.9	.76	.....
9.....	8.3	3.2	2.4	91.8	8.2	1.74	.....	43.2	39.6	3.6	.69	.....

[a] On dry-matter basis.

TABLE I.—*Average length, lateral diameter, dorsoventral diameter, and percentage and weight per kernel of dry matter, water, nitrogen, and ash in kernels of Hannchen barley sampled at 24-hour intervals from eight plots variously irrigated at the Aberdeen Substation, Aberdeen, Idaho, in 1917*—Continued

PLOT 3

Date.	Length.	Lateral diameter.	Dorsoventral diameter.	Percentage of weight per kernel.				Weight per kernel.				
				Dry matter.	Water.	Nitrogen.[a]	Ash.[a]	Wet weight.	Dry matter.	Water.	Nitrogen.	Ash.
	Mm.	*Mm.*	*Mm.*					*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
July 17	4.3	1.5	0.9	21.3	78.7	3.41	5.90	5.2	1.1	4.1	0.04	0.06
18	6.1	1.6	.9	21.6	78.4	2.37	4.00	8.2	1.8	6.4	.04	.07
19	7.3	1.7	1.1	22.5	77.5	2.44	3.80	10.9	2.5	8.4	.06	.08
20	8.4	2.0	1.3	23.3	76.7	2.24	3.37	15.9	3.7	12.2	.09	.11
21	9.1	2.2	1.5	23.9	76.1	2.18	4.67	18.9	4.5	14.4	.09	.24
22	9.8	2.5	1.6	25.9	74.1	2.56	3.61	24.1	6.3	17.8	.15	.25
23	9.3	2.5	1.7	26.8	73.2	2.14	3.87	26.4	7.1	19.3	.17	.25
24	9.8	3.0	2.0	31.5	68.5	2.24	3.79	35.3	11.2	24.1	.28	.38
25	9.8	3.1	2.0	31.2	68.8	2.06	4.02	35.5	11.0	24.5	.23	.43
26	9.9	3.2	2.1	35.3	64.7	.....	3.17	38.9	13.7	25.2	.....	.43
27	9.8	3.3	2.2	37.6	62.4	2.07	2.20	44.1	16.6	27.5	.35	.36
28	9.7	3.4	2.3	36.4	63.6	2.04	2.60	45.9	16.7	29.2	.34	.43
29	9.6	3.5	2.3	40.8	59.2	2.00	2.30	45.8	18.7	27.1	.39	.41
30	9.1	3.6	2.5	41.0	59.0	2.00	.....	51.7	21.6	30.1	.45	.....
31	8.9	3.6	2.5	41.8	58.2	1.96	2.14	52.7	22.0	30.7	.42	.48
Aug. 1	8.8	3.6	2.5	43.6	56.4	2.07	2.08	53.2	23.1	30.1	.46	.50
2	8.7	3.6	2.6	48.1	51.9	2.23	2.06	53.8	25.8	28.0	.58	.53
3	8.7	3.6	2.6	49.1	50.9	2.41	2.17	56.0	27.5	28.5	.67	.59
4	8.8	3.7	2.7	49.1	50.9	2.11	2.14	57.5	28.2	29.3	.61	.59
5	8.6	3.6	2.6	53.0	47.0	2.17	1.93	56.3	29.8	26.5	.64	.58
6	8.6	3.6	2.6	55.3	44.7	2.13	2.04	55.5	30.6	24.9	.65	.63
7	8.4	3.5	2.5	56.8	43.2	2.36	1.96	53.7	30.4	23.3	.70	.62
8	8.2	3.4	2.5	56.3	43.7	2.55	1.71	51.1	28.8	22.3	.64	.55

PLOT 4

July 20	8.6	1.9	1.3	24.4	75.6	2.77	3.9	15.5	3.8	11.7	0.10	0.15
21	8.9	2.0	1.3	24.3	75.7	3.08	4.8	16.1	3.9	12.2	.12	.19
22	9.7	2.5	1.6	27.2	72.8	2.71	3.6	23.6	6.4	17.2	.20	.20
23	9.7	2.8	1.8	28.3	71.7	2.65	3.2	27.9	7.9	20.0	.20	.27
24	9.5	2.9	1.9	29.5	70.5	2.38	3.3	31.8	9.4	22.4	.24	.28
25	9.9	3.1	2.1	33.3	66.7	1.15	3.8	37.9	12.6	25.3	.14	.49
26	9.8	3.2	2.1	34.2	65.8	2.11	3.2	34.4	13.5	20.9	.27	.45
27	9.7	3.4	2.2	34.3	65.7	2.39	3.0	41.3	14.1	27.2	.34	.42
28	9.9	3.4	2.3	36.1	63.9	2.19	2.9	44.3	16.0	28.3	.34	.48
29	9.9	3.4	2.3	39.9	60.1	1.39	3.2	46.1	18.6	27.5	.26	.....
30	8.8	3.5	2.4	42.2	57.8	2.12	2.3	47.1	19.8	27.3	.42	.46
31	9.0	3.6	2.5	42.8	57.2	2.25	2.4	52.6	22.5	30.1	.53	.51
Aug. 1	8.8	3.6	2.5	46.2	53.8	2.31	2.0	52.2	24.1	28.1	.55	.48
2	9.0	3.7	2.6	46.9	53.1	2.31	2.3	58.2	27.3	30.9	.63	.63
3	8.7	3.7	2.6	49.8	50.2	2.43	2.0	56.3	28.0	28.3	.69	.55
4	8.6	3.5	2.6	50.0	50.0	2.35	2.2	52.7	26.4	26.3	.59	.60
5	8.7	3.6	2.7	51.8	48.2	2.62	2.0	54.8	28.4	26.4	.73	.58
6	8.5	3.6	2.6	53.7	46.3	2.90	2.3	54.1	29.1	25.0	.75	.75
7	8.6	3.6	2.6	55.7	44.3	1.83	2.0	54.7	30.5	24.2	.56	.61
8	8.3	3.5	2.5	58.1	41.9	2.75	1.7	50.6	29.4	21.2	.81	.50

PLOT 5

July 23	9.5	2.9	1.8	32.1	67.9	1.96	3.3	29.9	9.6	20.3	0.18	0.33
24	9.7	2.9	1.8	31.0	69.0	2.87	2.8	30.2	9.4	20.8	.26	.27
25	9.7	3.2	2.1	34.6	65.4	2.31	2.1	37.4	13.0	24.4	.26	.31
26	9.6	3.1	1.9	33.3	66.7	2.63	2.1	34.8	11.6	23.2	.27	.28
27	9.7	3.3	2.1	35.1	64.9	2.55	3.2	40.1	14.1	26.0	.39	.41
28	9.7	3.4	2.2	36.8	63.2	2.82	.....	43.3	15.9	27.4	.42	.....
29	9.5	3.4	2.2	41.4	58.6	.....	2.2	44.6	18.5	26.1	.....	.40
30	8.8	3.5	2.4	43.8	56.2	2.62	2.4	48.8	21.4	27.4	.52	.55
31	8.7	3.4	2.4	44.1	55.9	2.13	2.1	48.0	21.1	26.9	.43	.47
Aug. 1	8.8	3.5	2.4	44.5	55.5	1.96	2.3	50.6	22.5	28.1	.41	.55
2	8.4	3.5	2.5	48.5	51.5	2.28	2.2	49.6	24.1	25.5	.54	.54
3	8.4	3.6	2.4	47.8	52.2	2.84	2.1	48.5	23.2	25.3	.63	.51
4	8.6	3.5	2.5	48.9	51.1	2.58	2.1	51.6	25.2	26.4	.67	.52
5	8.3	3.6	2.5	51.7	48.3	2.56	2.2	51.8	26.8	25.0	.66	.61
6	8.6	3.5	2.6	52.2	47.8	2.88	1.7	51.9	27.0	24.9	.78	.46
7	8.4	3.5	2.5	56.1	43.9	2.88	2.5	53.4	29.9	23.5	.85	.76
8	8.1	3.4	2.6	54.9	45.1	2.52	1.9	50.0	27.5	22.5	.73	.50
9	8.1	3.4	2.5	54.7	45.3	2.99	1.9	50.6	27.7	22.9	.90	.48

[a] On dry-matter basis.

TABLE I.—*Average length, lateral diameter, dorsoventral diameter, and percentage and weight per kernel of dry matter, water, nitrogen, and ash in kernels of Hannchen barley sampled at 24-hour intervals from eight plots variously irrigated at the Aberdeen Substation, Aberdeen, Idaho, in 1917*—Continued

PLOT 6

Date.	Length.	Lateral diameter.	Dorsoventral diameter.	Percentage of weight per kernel.				Weight per kernel.				
				Dry matter.	Water.	Nitrogen.[a]	Ash.[a]	Wet weight.	Dry matter.	Water.	Nitrogen.	Ash.
	Mm.	*Mm.*	*Mm.*					*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
July 26	9.4	3.0	1.8	37.5	62.5	2.81	2.2	32.4	12.2	20.2	0.25	0.26
27	9.8	3.3	2.1	38.9	61.1	2.21	2.2	41.1	16.0	25.1	.34	.36
28	9.8	3.3	2.2	39.2	60.8	2.22	2.1	42.7	16.7	26.0	.37	.35
29	9.7	3.3	2.2	40.2	59.8	2.68	1.9	40.7	16.3	24.4	.44	.31
30	9.0	3.5	2.3	41.7	58.3	2.37	1.9	46.8	19.5	27.3	.45	.38
31	8.8	3.4	2.3	37.4	62.6	3.79	2.4	44.9	16.8	28.1	.50	.49
Aug. 1	8.6	3.3	2.2	47.5	52.5	2.27	1.9	42.0	20.0	22.0	.47	.36
2	8.6	3.4	2.4	49.1	50.9	2.45	1.9	51.1	25.1	26.0	.59	.50
3	8.3	3.4	2.3	49.8	50.2	2.88	1.6	44.1	22.0	22.1	.58	.38
4	8.5	3.4	2.4	49.0	51.0	2.39	3.1	46.7	22.9	23.8	.61	.63
5	9.0	3.6	2.6	51.8	48.2	2.83	2.2	55.9	28.9	27.0	.74	.70
6	8.4	3.4	2.4	51.9	48.1	2.38	2.6	47.3	24.6	22.7	.65	.57
7	8.3	3.4	2.4	55.1	44.9	2.79	1.6	48.0	26.4	21.6	.78	.40
8	8.4	3.4	2.5	55.4	44.6	.....	1.7	48.2	26.7	21.5	.....	.46

PLOT 7

July 29	9.3	3.2	2.0	43.6	56.4	3.04	2.0	37.8	16.5	21.3	0.51	0.32
30	8.8	3.3	2.2	46.1	53.9	2.37	2.1	42.1	19.4	22.7	.45	.42
31	8.6	3.4	2.4	46.6	53.4	2.43	1.8	45.6	21.2	24.4	.52	.38
Aug. 1	8.8	3.3	2.2	47.9	52.1	2.63	2.1	44.6	21.3	23.3	.57	.44
2	8.6	3.3	2.3	48.0	52.0	3.01	1.9	42.5	20.4	22.1	.62	.39
3	8.5	3.3	2.3	50.6	49.4	.....	1.7	43.8	22.9	20.9	.....	.39
4	8.7	3.4	2.3	50.1	49.9	2.77	1.7	45.3	22.7	22.6	.59	.41
5	8.5	3.4	2.3	51.1	48.9	2.61	1.7	46.0	23.5	22.5	.66	.37
6	8.3	3.2	2.3	53.1	46.9	2.81	2.2	42.2	22.4	19.8	.62	.50
7	8.2	3.2	2.3	55.2	44.8	2.55	2.2	44.1	24.4	19.7	.68	.49
8	8.1	3.2	2.4	57.3	42.7	2.53	1.6	45.7	26.1	19.6	.66	.42

PLOT 8

July 16	3.7	1.4	0.9	22.2	77.8	2.74	9.6	4.0	0.9	3.1	0.02	0.09
17	4.7	1.5	.8	22.0	78.0	2.40	4.4	5.9	1.3	4.6	.03	.07
18	6.0	1.6	.9	22.2	77.8	2.36	6.8	7.3	1.6	5.7	.03	.14
19	8.0	1.8	1.1	23.7	76.3	2.05	4.5	14.0	3.3	10.7	.07	.13
20	8.6	2.0	1.3	24.6	75.4	2.40	3.8	15.7	3.9	11.8	.09	.16
21	9.5	2.1	1.5	25.3	74.7	1.87	3.2	19.5	4.9	14.6	.09	.16
22	9.6	2.3	1.6	26.6	73.4	2.62	3.5	22.6	6.0	16.6	.16	.21
23	9.6	2.5	1.8	29.9	70.1	2.35	3.1	27.2	8.1	19.1	.20	.24
24	9.6	3.0	1.9	34.9	65.1	2.37	2.2	33.4	11.7	21.7	.29	.24
25	9.7	2.9	1.9	36.2	63.8	2.10	3.0	34.9	12.7	22.2	.31	.32
26	9.8	3.1	2.0	38.8	61.2	2.19	2.5	37.0	14.4	22.6	.32	.36
27	9.7	3.2	2.1	39.4	60.6	2.32	2.3	40.6	16.0	24.6	.35	.38
28	9.7	3.2	2.0	40.8	59.2	2.84	2.6	37.9	15.5	22.4	.43	.41
29	9.8	3.3	2.1	44.2	55.8	2.41	2.3	40.4	17.9	22.5	.44	.40
30	8.8	3.3	2.2	46.7	53.3	2.85	2.5	42.3	19.8	22.5	.56	.49
31	8.8	3.4	2.2	46.8	53.2	2.49	2.2	43.9	20.5	23.4	.51	.45
Aug. 1	8.5	3.3	2.2	48.9	51.1	2.91	2.1	41.4	20.2	21.2	.62	.40
2	8.4	3.2	2.3	50.9	49.1	2.56	2.0	43.5	22.1	21.4	.59	.43
3	8.6	3.2	2.2	53.1	46.9	2.86	1.9	42.4	22.4	20.0	.68	.40
4	8.4	3.2	2.2	54.0	46.0	2.86	1.6	41.6	22.4	19.2	.65	.35
5	8.4	3.0	2.1	62.6	37.4	2.69	1.9	34.6	21.6	13.0	.61	.39
6	8.3	2.9	2.0	64.2	35.8	3.16	2.0	33.0	21.2	11.8	.67	.42

[a] On dry-matter basis.

The length of kernel is not a very satisfactory index of development. As has been previously shown, the full length is attained within a few days after flowering and remains nearly constant during the remainder of the period of development. As the grain ripens, however, the loss

of water and consequent turgor result in a slight decrease in length. The average lengths of kernels from the 8 plots are shown in figure 1. These are averages of daily measurements until July 23. After that date they are the averages of two days, as the length had become so nearly constant as to make daily averages of no advantage. The drop in length on July 30 is due to a change in the method of measurement.

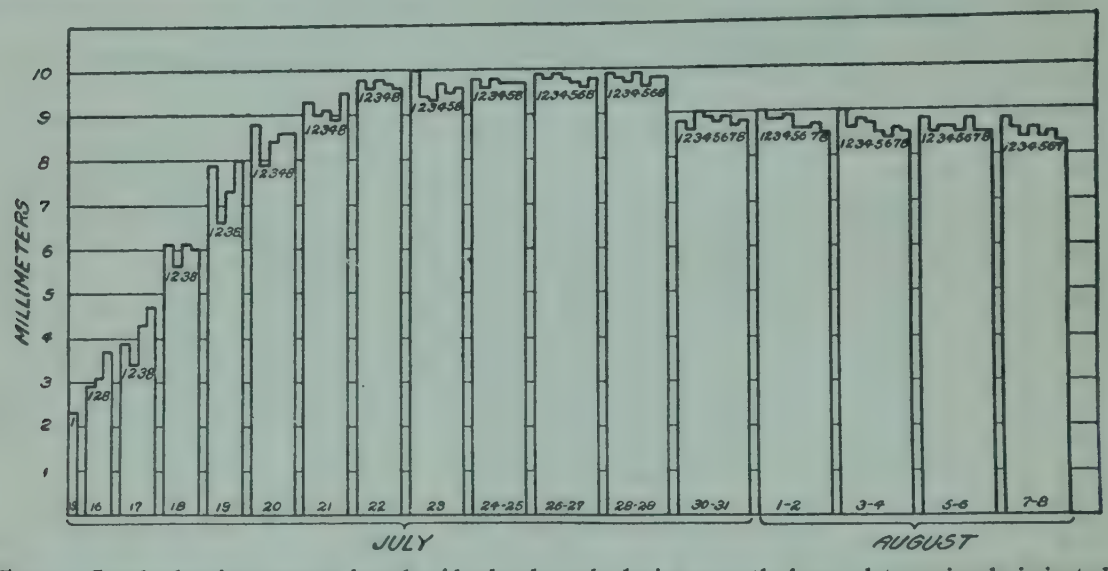


FIG. 1.—Graph showing average length of barley kernels during growth, from plots variously irrigated.

After this date it was possible to measure the kernels without including the ovary tissues, which had then been largely resorbed. Previous to this date the measurements include ovary tissue about 1 mm. in length.

Figure 1 shows two things. Plot 1, which, because of the irrigation designed to maintain satisfactory growth, exceeded the other plots in size of kernels, possessed no advantage at the beginning. Its kernels

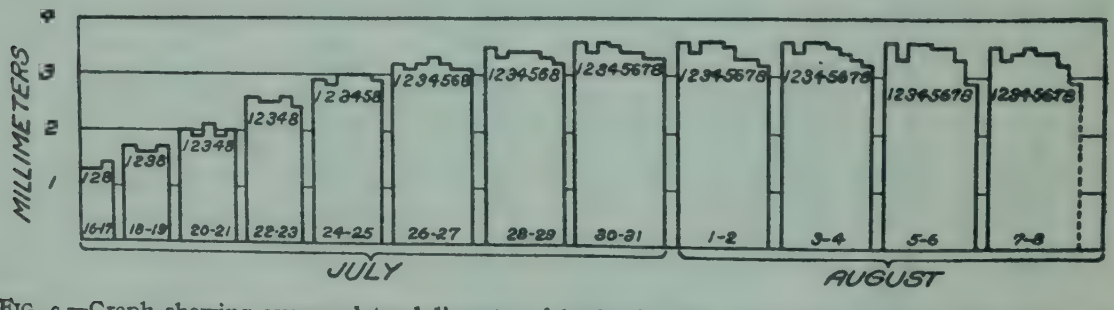


FIG. 2.—Graph showing average lateral diameter of barley kernels during growth, from plots variously irrigated.

were of no greater size than those of the other plots. On the other hand, it is apparent that the kernels from plots 7 and 8 never attained the length of the kernels from plot 1 and that the shrinking at maturity occurred earlier in these plots.

The maximum lateral diameter of the kernel was not attained until the last of July. As maturity approached, there was slight decrease. In figure 2 the lateral measurements of kernels from plot 2 are shown to

be considerably lower than those of kernels from plot 3 or 4. A part of this may be due to soil conditions, but a part is due also to the greater culm formation. The irrigation of July 14 on plot 2 stimulated stooling to such an extent that more spikes were produced in this plot than in any of those irrigated later. As it received no further irrigation, the competition was more intense and the plants suffered from drouth toward maturity. As maturity approached, the diameters of kernels from plots 6 and 7 were greater than those of kernels from plot 8, showing benefit from late irrigation.

The dorsoventral diameter (fig. 3) does not reach its maximum until just before maturity. There was little difference between these measurements of kernels from the different plots until July 27. After this date there is an evident relation between the dorsoventral diameter and the date of the application of water, excepting in plot 2, which was somewhat abnormal. The kernels from plots 3 and 4 are almost equal to those of plot 1. These kernels, it will be remembered, are from selected

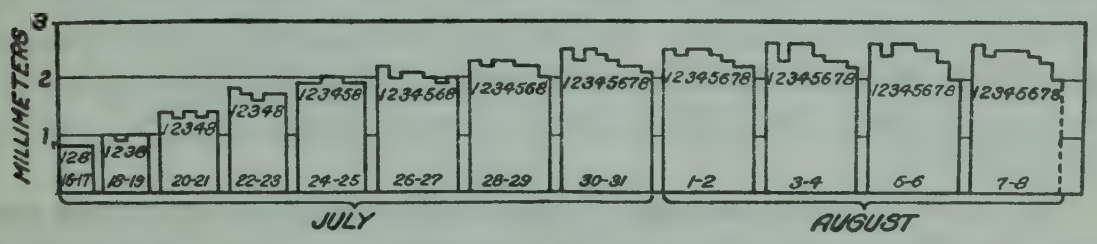


FIG. 3.—Graph showing dorsoventral diameters of barley kernels during growth, from plots variously irrigated.

spikes, the total number of spikes being much less in those plots. The plants in plots 6 and 7 were able to use the water applied as late as July 26 and 29. They showed an increase in the dorsoventral diameter of the kernels as compared with those from plot 8, and the maturation was delayed by the application.

The average weights per kernel of all intermediate plots fell between those of plot 1 and plot 8. The data on weights present one surprising feature. The wet weight per kernel is an earlier index of final size of kernel than is the dry weight. In figures 4 to 10 it appears that the moisture of the irrigation of June 23 was sufficient to maintain a maximum growth on plot 8 until July 27. After that date, the wet weight of kernels from plot 8 was less than that of those from plots 1 to 4. In figures 11 to 17 it will be seen that this is not the case with the dry matter. The weight of dry matter in kernels from plot 8 is equal to that of those from plots 2, 3, and 4 until July 30. The cause of this is not evident. It would appear that the size of kernel is determined before the rate of deposit of material is checked. In figure 18, the water content of the kernels from plot 8 is seen to have reached the maximum by July 27. This is not true in any other plot. This means that whatever change takes

place at this time occurs under the conditions of plot 8 two or three days earlier than in plots 1 to 4. This change may mark the end of the addition of new cells, the abandonment of the central cells for active deposition, the entering of the second stage of starch formation where numerous new grains are started, or it may be some fundamental change the significance of which is not yet recognized.

The kernels from plots 5, 6, and 7 show departure from the rate of deposit in those of plot 8 at later dates. There is a departure in all cases; and even in plot 7, irrigated as late as July 29, the kernels are significantly greater in size and their maturation date is postponed by the irrigation.

The status of the water supply on succeeding days is more apparent in figure 18. The course of the water content of kernels from plot 1 is here seen to be very regular. From this plot four samples were taken instead of two, and the more favorable irrigation caused a more uniform development in the plants. If some allowance is made for plot 2, the effect of time of irrigation is quite apparent. By August 1, plot 2 has begun to suffer from lack of water, while 3 and 4 are enjoying the full benefit of a sufficient supply. By August 7, maturation is well advanced, the kernels from plot 5 being equal to those from plots 3 and 4 in water content and those from plots 6 and 7 showing obvious benefit from the late irrigation.

The trend of the percentage of ash is very uniform. As can be seen from the table, there is a gradual daily decrease from about 8 per cent at flowering to less than 1 per cent at maturity. When expressed in milligrams per kernel, the ash content bears a direct though not exact relationship to quantity of the water used. This is apparent in figure 19, where the ash per kernel is averaged in 3-day periods. There is some evidence that the application of water retards the deposit of ash for two or three days following irrigation. The curves of July 31 and August 6 are strongly suggestive of the availability of water. It is to be supposed that plots 2 to 4 would use more water than those irrigated later, as they had the use of water for a longer period. Plots 3 and 4 received much less water than plot 1, but they contained fewer culms and the water per culm seems to have been sufficient for an equal deposit of ash.

The nitrogen determinations were not satisfactory. As may be seen in the table, the percentages varied from day to day more than seems natural. At first this is due to extremely small samples, but the variation is just as great at maturity. No definite conclusions can be drawn, but from figure 20 it appears that a plentiful water supply may hinder the deposit of nitrogen. As much nitrogen apparently is deposited in the kernels in plots suffering for water as in those amply provided with it. Of course the percentage of nitrogen is higher in the smaller kernels.

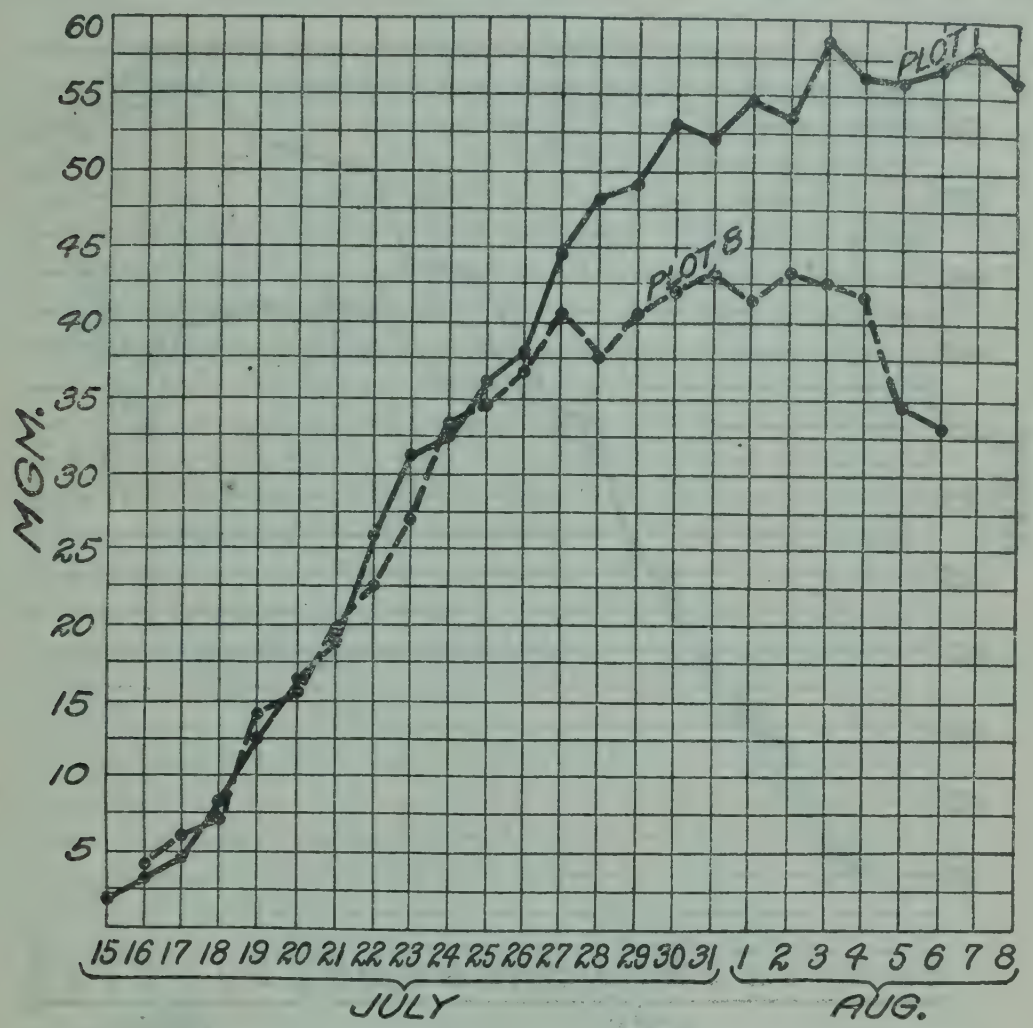


FIG. 4.—Graph showing wet weight in milligrams of barley kernels from plots 1 and 8.

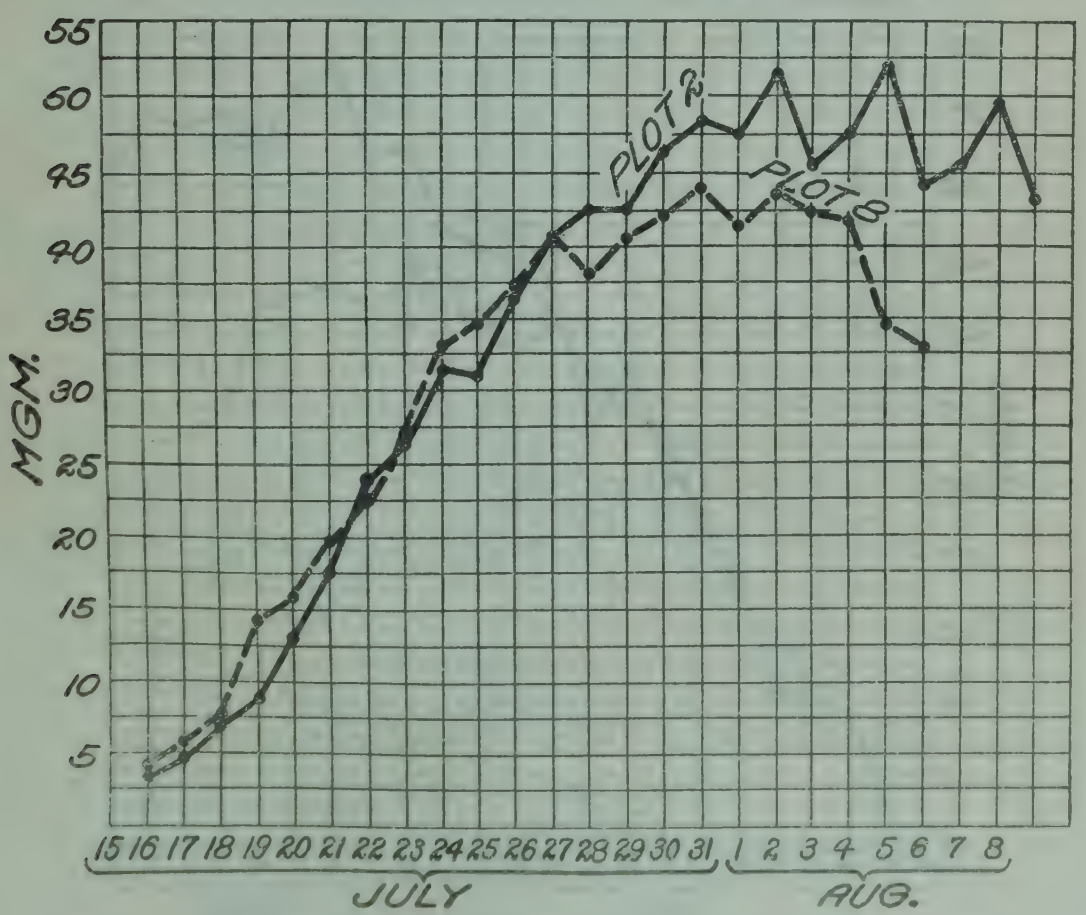


FIG. 5.—Graph showing wet weight in milligrams of barley kernels from plots 2 and 8.

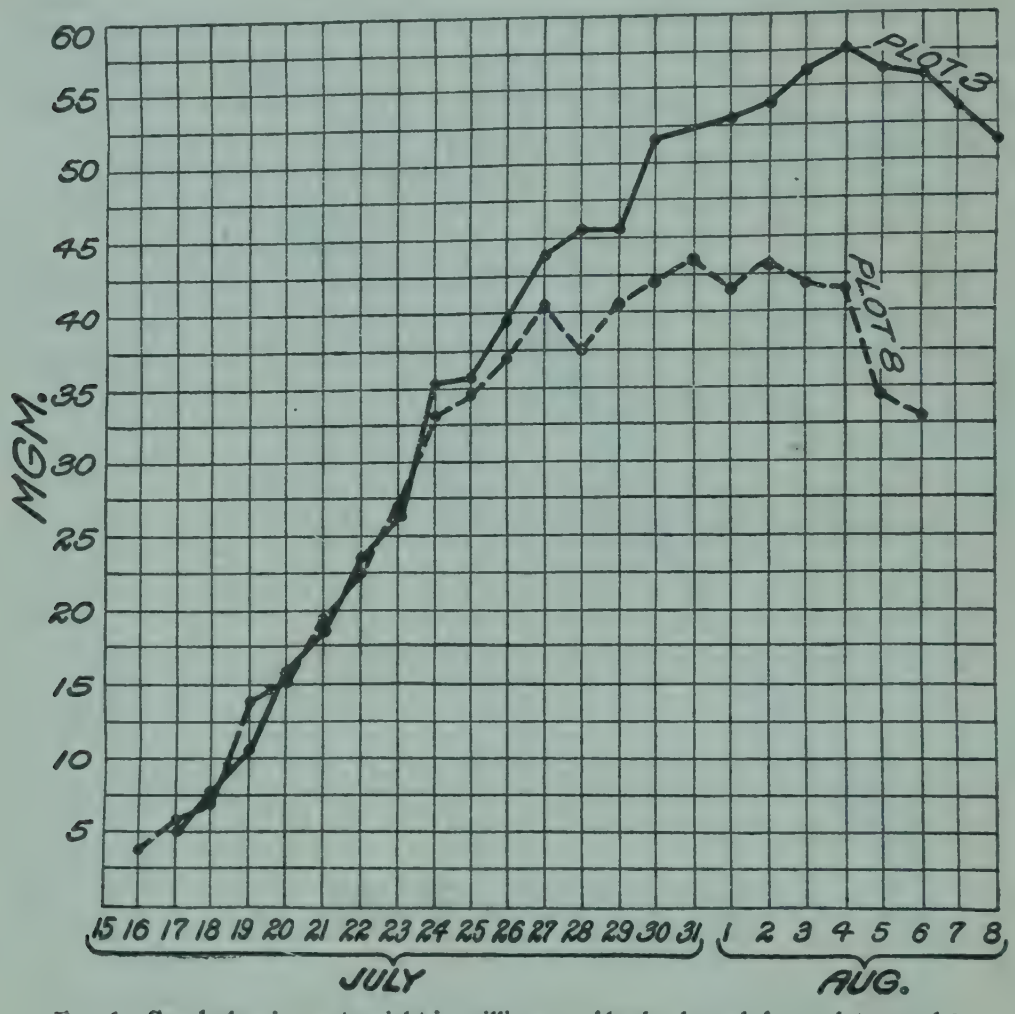


FIG. 6.—Graph showing wet weight in milligrams of barley kernels from plots 3 and 8.

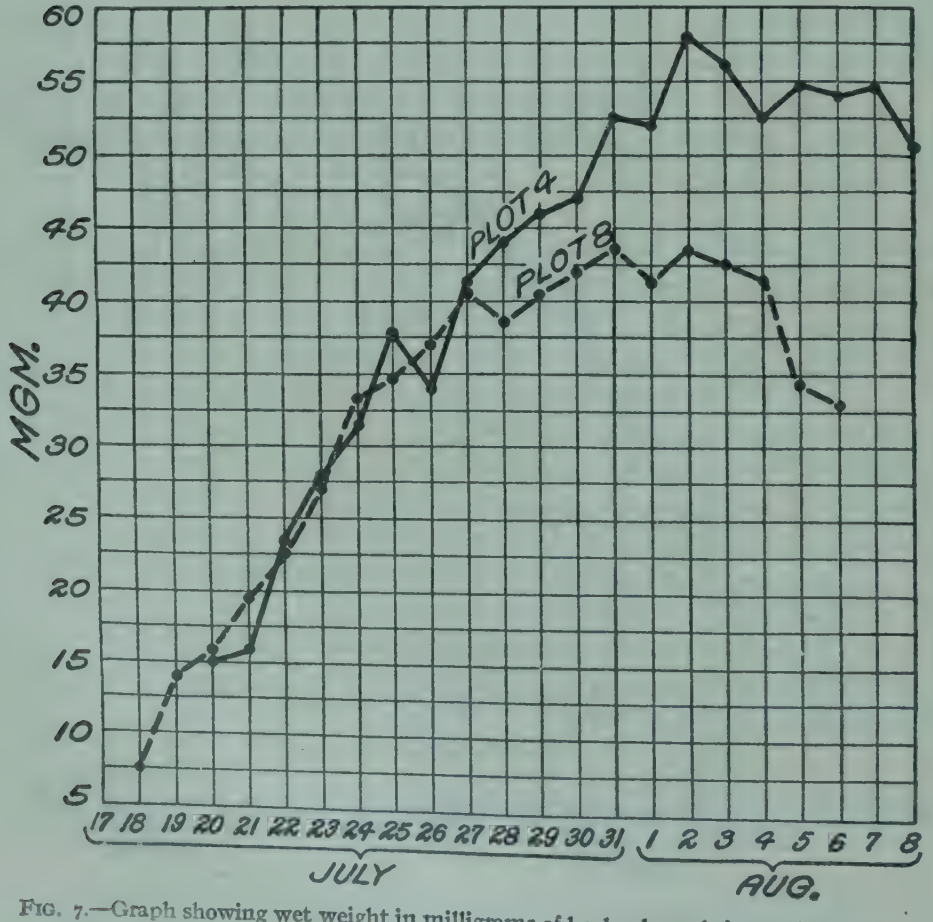


FIG. 7.—Graph showing wet weight in milligrams of barley kernels from plots 4 and 8.

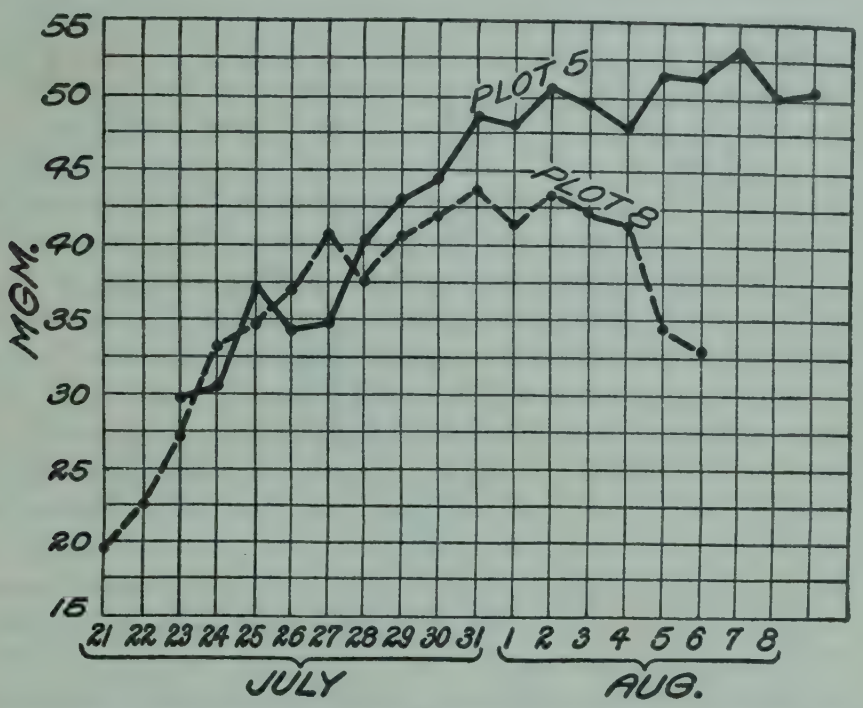


FIG. 8.—Graph showing wet weight in milligrams of barley kernels from plots 5 and 8.

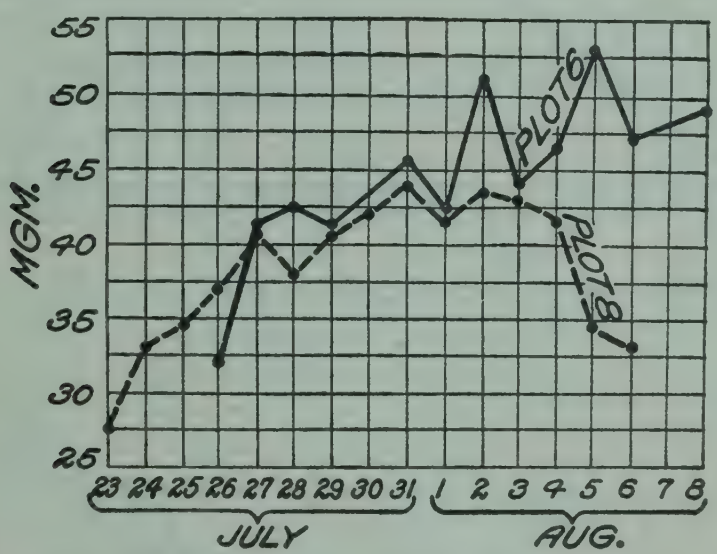


FIG. 9.—Graph showing wet weight in milligrams of barley kernels from plots 6 and 8.

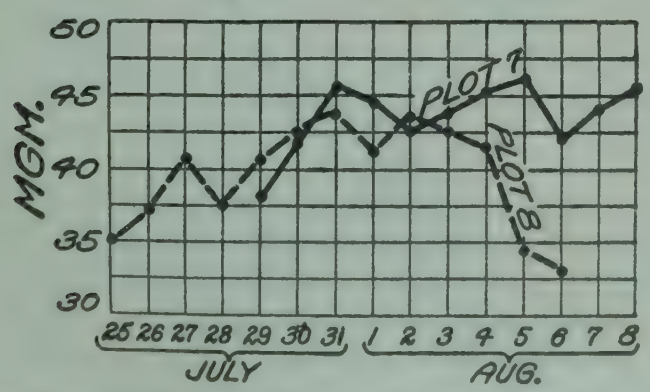


FIG. 10.—Graph showing wet weight in milligrams of barley kernels from plots 7 and 8.

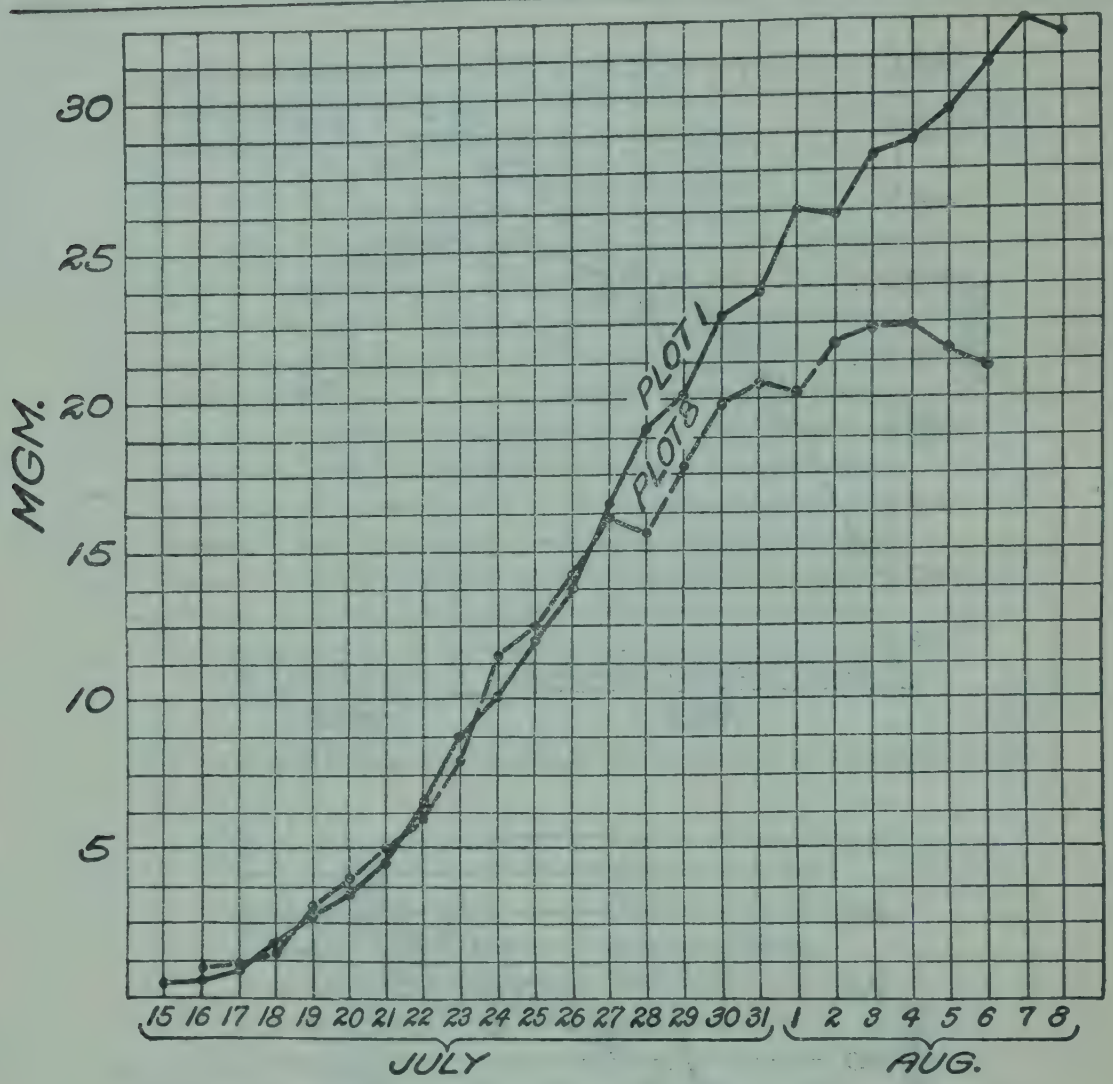
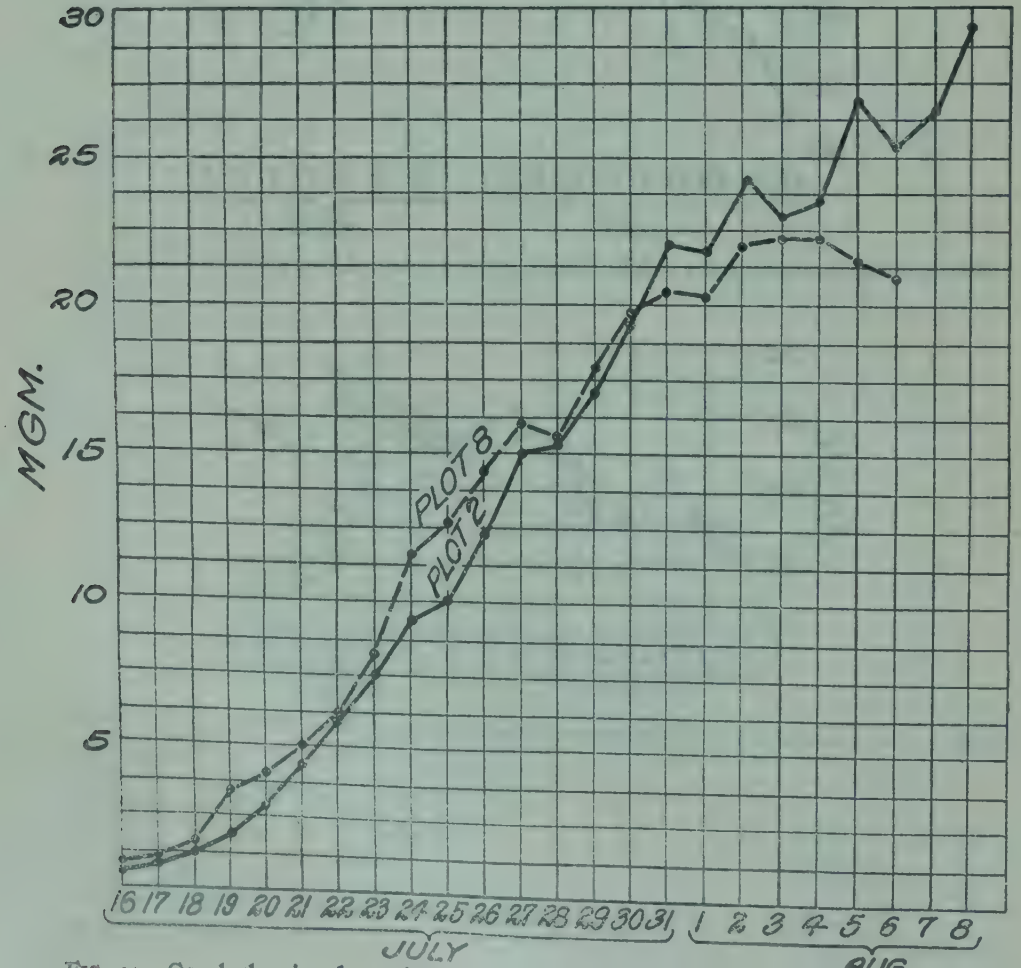


FIG. 11.—Graph showing dry weight in milligrams of barley kernels from plots 1 and 8.



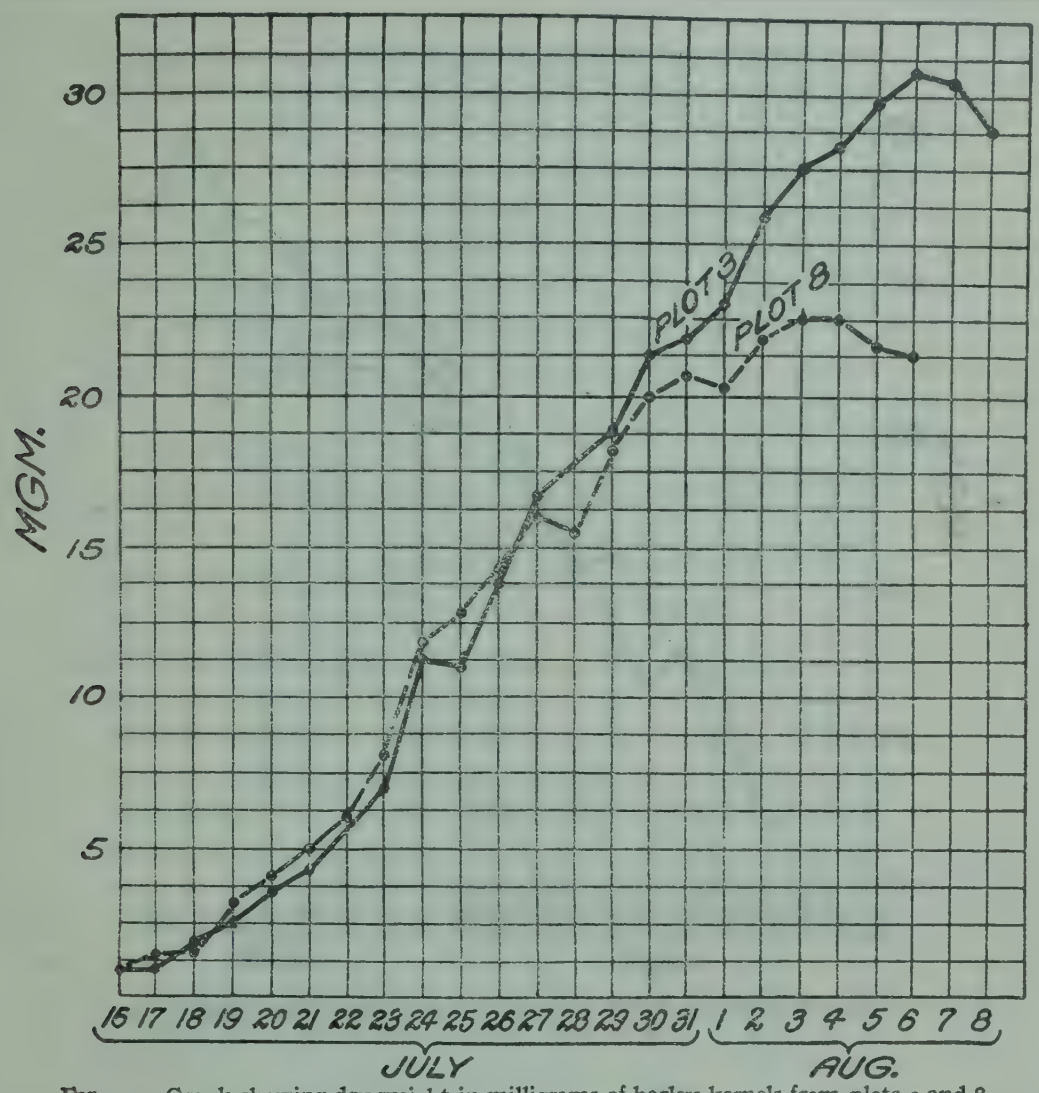
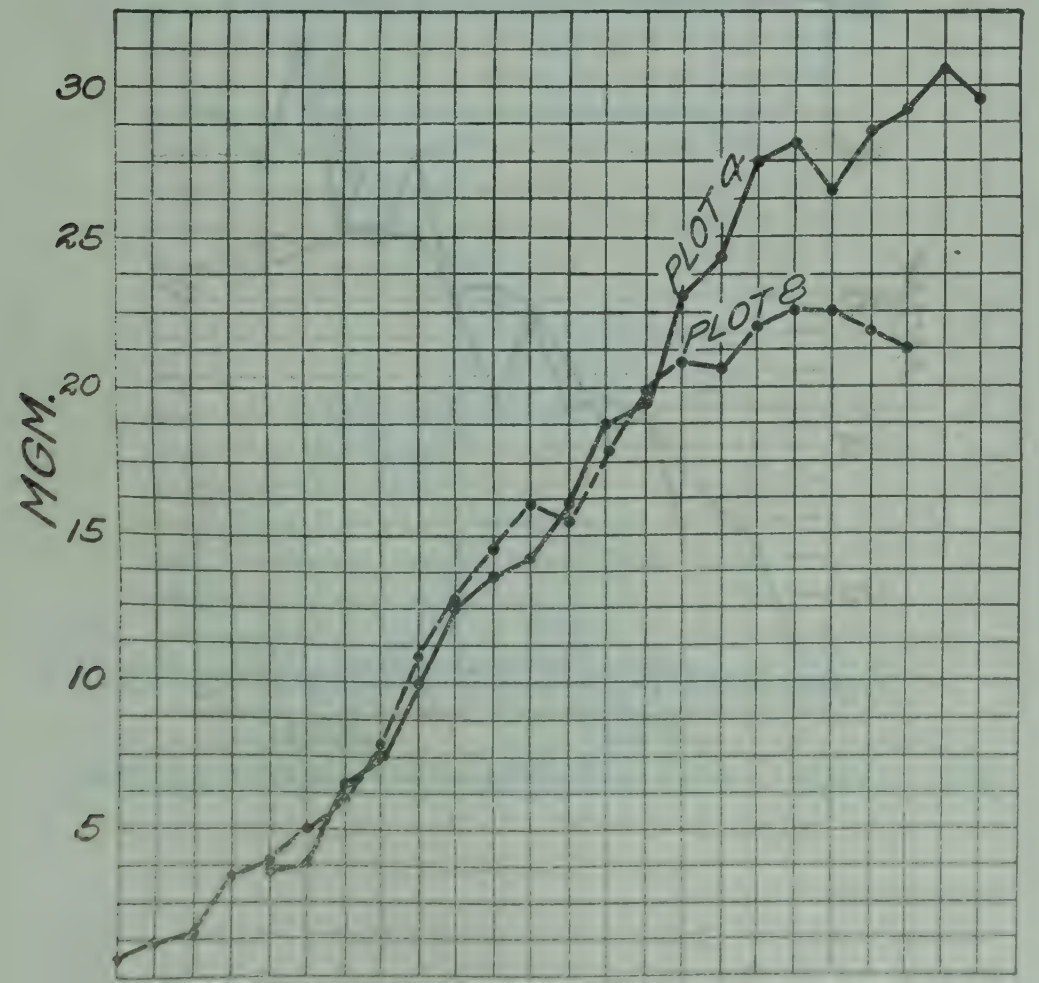


FIG. 13.—Graph showing dry weight in milligrams of barley kernels from plots 3 and 8.



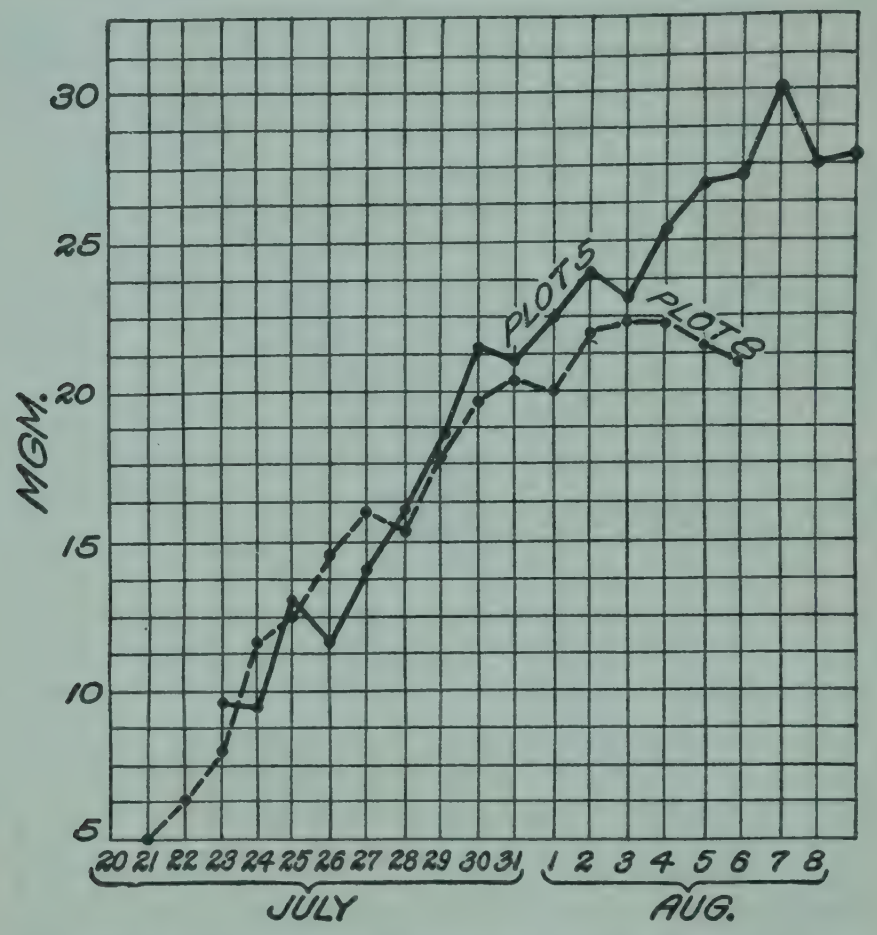


FIG. 15.—Graph showing dry weight in milligrams of barley kernels from plots 5 and 8.

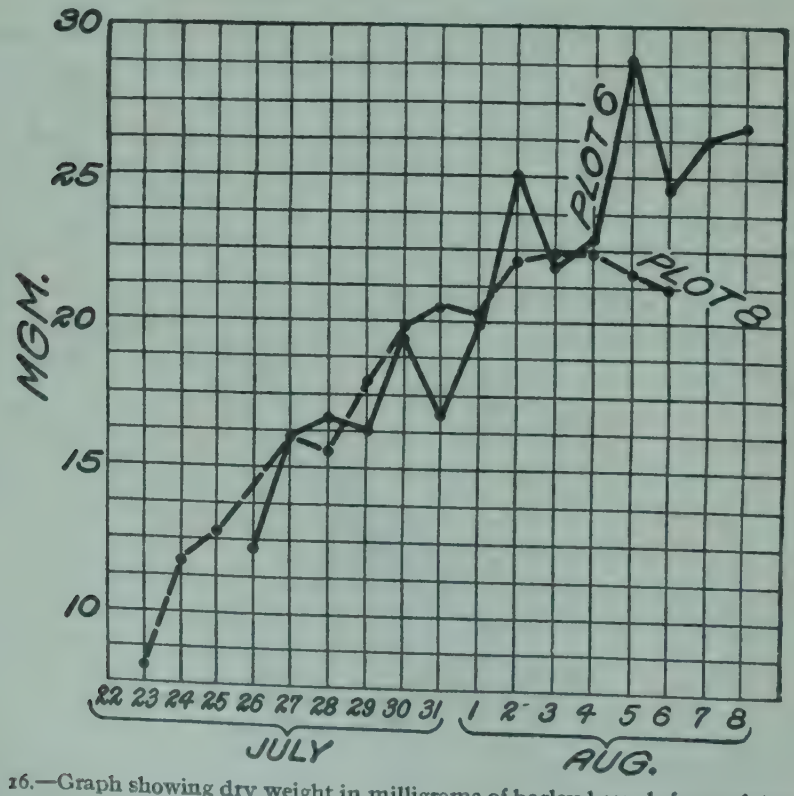


FIG. 16.—Graph showing dry weight in milligrams of barley kernels from plots 6 and 8.

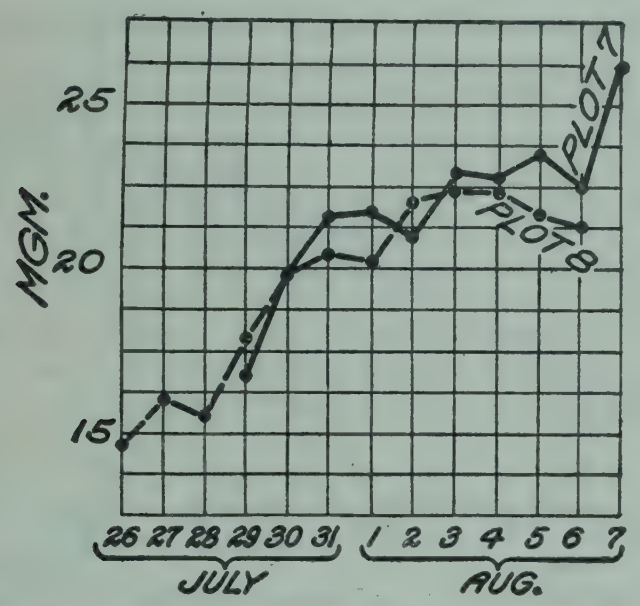


FIG. 17.—Graph showing dry weight in milligrams of barley kernels from plots 7 and 8.

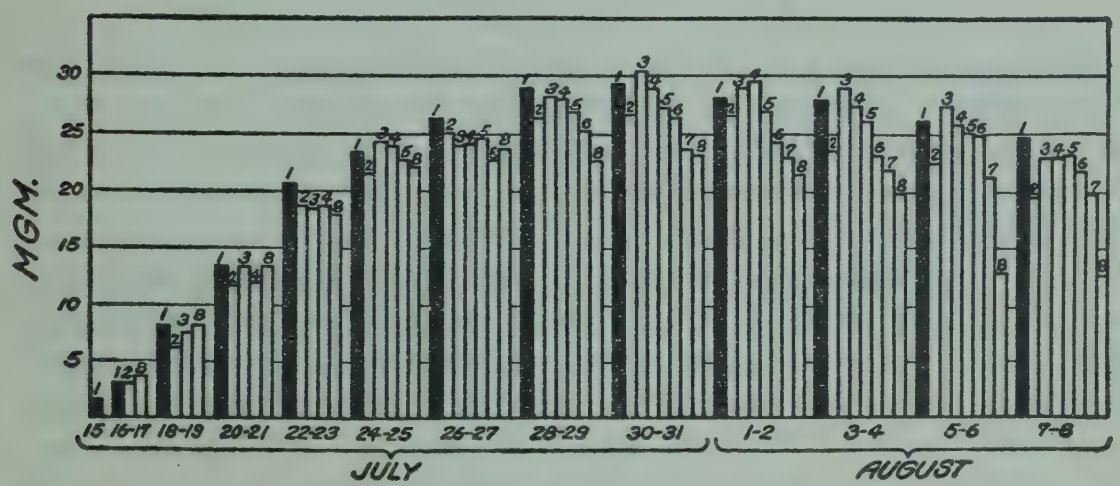


FIG. 18.—Graph showing water content of barley kernels from plots variously irrigated. Data shown are in milligrams per kernel and are averages of daily measurements during each 2-day period.

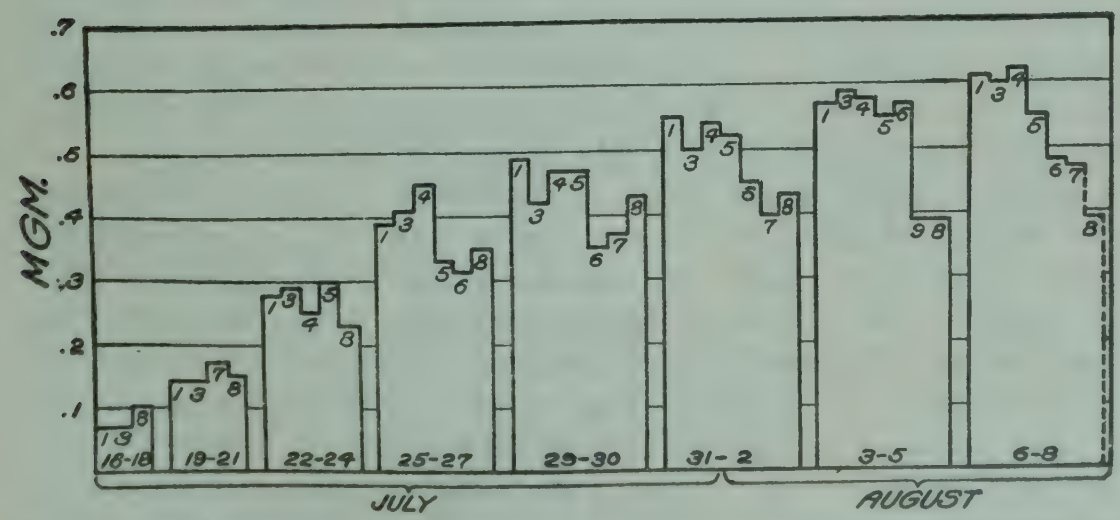


FIG. 19.—Graph showing ash content in barley kernels from plots variously irrigated. Data shown are in milligrams per kernel and are averages of daily measurements during each 3-day period.

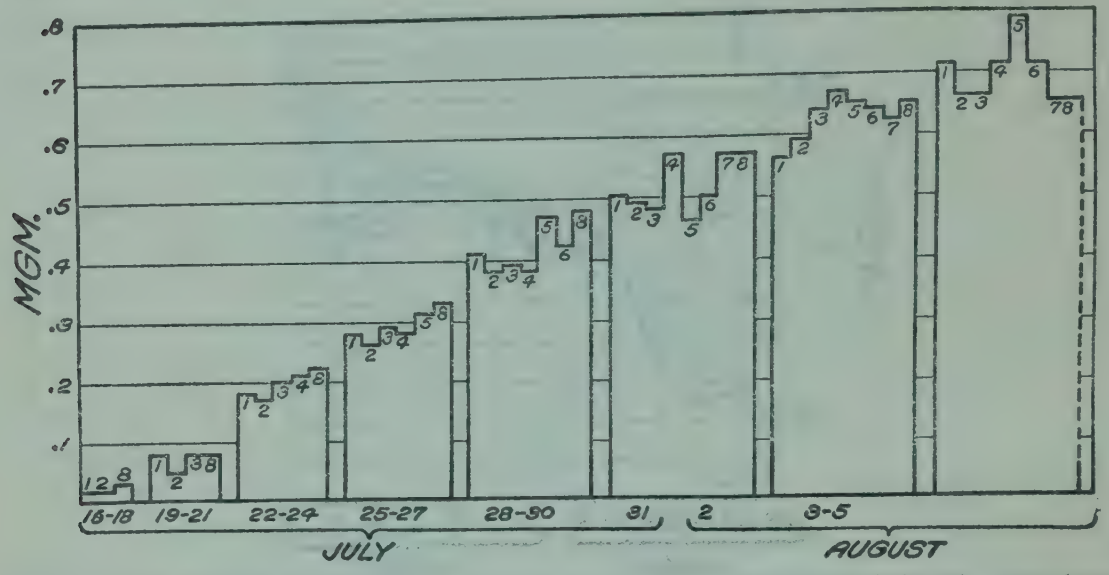


FIG. 20.—Graph showing nitrogen content of barley kernels from plots variously irrigated. Data shown are in milligrams per kernel and are averages of daily measurements during each 3-day period.

DISCUSSION OF RESULTS

The data presented show the course of development of kernels for different plots. Some facts, however, they do not and can not show. They show, for instance, that the development of kernels in plot 2 is not so great as in plots 3 and 4. They do not show that a part of this is due to the extra culm formation on this plot as compared with those irrigated later. It is probable that the soil of plot 2 was somewhat below the average; but, in addition to this, there was a heavy development of culms which caused the water to be exhausted rather early in growth. The foliage of this plot showed that its plants were suffering from lack of water by July 30. On plots 3 to 8, the number of culms was much less, many of the secondary culms being arrested before they drew on the water supply. On the plots irrigated late in the series, wolf plants developed which by their extra development used more than an average amount of water and caused some irregularities. These are apparent in the data from plot 5. The plot yields are not given, as the study was not planned to include so many factors as are involved in the total yield. The kernel weights bear no necessary relation to the yield per plot.

The major point of the study is conclusive. Plants, even though suffering from drouth, are able to utilize water in late applications. On plots 6 and 7 the plants were well along toward maturity when water was applied. The advanced stage of maturity is not shown by the growth curves. The sterile lateral spikelets of plants in plot 8 had lost their green color by August 1—that is, plot 7 had been irrigated just before the actual abandonment of tissue in the spike took place through drying or maturity. By August 3 the color was disappearing on the side florets of plants in plot 2, which had begun to suffer from drouth a few days before. By August 4 the color was apparent only around the furrow of the kernels from this plant, and the lateral florets of plot 3 were

losing color. The plants on plot 3 had exhausted their water supply to the point of wilting badly. Those on plot 4 were affected slightly. This is apparent in the various graphs, but is more significant when correlated with the field observation.

The ripening was not uniform. On most of the plots the bases of the spikes were inclosed in the leaf sheaths. The kernels so inclosed did not ripen as soon as those which were exposed. The kernels from plot 8 were wrinkling by August 5 and therefore were ripe. The tip kernels from all plots were wrinkling by August 8, but the basal kernels in some cases were still adding material. Of the plots irrigated once after June 23, plot 5 was the last to ripen, having sufficient water and having been irrigated before maturity was as far advanced as in plot 7. The later maturity of plot 5 is apparent in figure 18.

The departure in the growth of the various plots came at about the critical stage pointed out in the paper on daily development—that is, 14 days after flowering. The course of the wet weights indicated that the ultimate size of the kernel was affected 3 days earlier. The nature of this change has not been investigated; but, as was suggested, it may be correlated with the stopping of the addition of new cells or with the entering upon the secondary phase of starch formation.

At Aberdeen, the deposit of dry matter continues until just before the grain wrinkles. Maturity at Aberdeen seems to occur at the earliest date possible without accident or drying. In other words, there is no prolonged period of maturation such as apparently takes place in districts where cold, wet weather occurs at ripening time.

CONCLUSIONS

At Aberdeen, Idaho, deposit of dry matter in the kernel continues until very near the point of absolute ripeness.

The plants are able to utilize water up to the date of full maturity. Late irrigation results in a greater activity and a later maturity.

A deficiency of water, even after the spikes are losing their color, results in checking the deposit of dry matter.

A deficiency of water earlier in the development of the kernel probably determines the size of the kernel, even before the rate of deposit of dry matter is checked.

LEAFROLL, NET-NECROSIS, AND SPINDLING-SPROUT OF THE IRISH POTATO[1]

By E. S. SCHULTZ, *Pathologist, Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture,* and DONALD FOLSOM, *Associate Plant Pathologist, Maine Agricultural Experiment Station*

INTRODUCTION

Potato (*Solanum tuberosum L.*) leafroll of the type that is transmissible but that has not been proved to be parasitic has been differentiated from other types by Appel (*3*)[2], Orton (*15*), Wortley (*24*), and others, and designated as phloem-necrosis by Quanjer (*16, 17*). It is considered by Quanjer (*17, p. 41*) to be an infectious disease caused by a virus or ultramicroscopic parasites. The absence of data concerning its cause and concerning natural and artificial means of its transmission has made it difficult to distinguish sharply between it and various other leafroll diseases of the potato and has resulted often in confusion and conflict in the literature (*3; 15, p. 3, 19, 25, 34; 17; 24*). The reviewing of this literature has been done previously (*15, 17, 18*) and is beyond the scope of this paper, which is concerned chiefly with symptoms and transmission.

SYMPTOMS

LEAFROLL

The principal macroscopic characteristics of the type of leafroll[3] considered in this paper include rolling, rigidity, brittleness, leathery texture, light green, yellowish, reddish, or purplish discoloration of the affected leaves; dwarfing of the vines, which usually remain erect and rigid; shortness of stolons; reduction in the number and size of tubers; and rigidity or woodiness of the tubers. Some of these characteristics may be absent at times. The rolling may appear upon the upper leaves alone or only upon the lower leaves or throughout the entire vine. It consists of an upward curving of the sides of each leaflet with the midrib at the bottom of the trough thus formed. (See Pl. 1, A; 2, A.) Its extent depends largely upon the time when infection occurred and upon the age of the affected plant. Plants infected relatively late in their development may show rolling in the upper leaves only, while those

[1] This paper is based upon investigations carried on as a cooperative project between the Office of Cotton, Truck, and Forage Crop Disease Investigations of the Bureau of Plant Industry, United States Department of Agriculture, and the Department of Plant Pathology of the Maine Agricultural Experiment Station. Unless otherwise indicated the work was performed in northeastern Maine in the vicinity of Presque Isle. The order of arrangement of the authors' names is not intended to indicate that one cooperating institution contributed more than the other to the results.

[2] Reference is made by number (italic) to "Literature cited," p. 78–80.

[3] Throughout this paper the term "leafroll" is employed as an abbreviation of "apparently nonparasitic, transmissible leafroll" and as a synonym of "phloem-necrosis."

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becoming infected from the tuber or when only a few centimeters tall show it in the lower leaves first, usually followed by rolling of progressively higher leaves later in the same season until most or all are rolled.

Some other diseases have characteristics in common with leafroll, but they can be distinguished from it. Leafroll is marked by rigidity or stiffness of the affected foliage (Pl. 1, A, B) in great contrast with the flaccid, wilted condition of the rolled leaves found on vines affected with wilt diseases. It causes the leaves to be rolled symmetrically instead of asymmetrically as on plants whose leaves, usually a few top ones, are injured by certain insects. It closely resembles when in the top alone the type of rolling found on plants affected with Rhizoctonia, but the effect of Rhizoctonia will be disclosed by examining more closely especially for the characteristic underground stem lesions. The type of leafroll caused by blackleg usually appears on the entire plant, accompanied by more pronounced yellowish discoloration, and disclosure of the blackened underground stem will distinguish this type further. A nonparasitic type of leafrolling resulting from excessive soil moisture resembles very closely the kind considered in this paper. However, in the former the leaves on the entire plant may become rolled at any time in the development of the vine, the foliage does not become as stiff and rigid, and the leafrolling is limited to segregated areas where soil moisture is excessive. Another nonparasitic type is caused by drouth conditions, but usually is limited to the lowest leaves on the side of the plant most exposed to sunlight and is accompanied by limpness rather than by rigidity of the leaves.

NET-NECROSIS

Orton first used the name "net-necrosis" in connection with an illustration (*15, Pl. II, fig. 2*). As described previously by other writers (*6, 7, 9*), the most apparent effect of this disease in the tuber is a discoloration of the fibrovascular bundles, originating at the rhizome scar. This is dark, varying from brown to black, and is in the form of a network (Pl. 2, B) which may extend throughout the whole tuber. The appearance of affected tubers in section is shown in Plate 3. The writers have seen net-necrosis occasionally absent in the stem end while pronounced in the bud-end half. While it is not difficult in severe cases to distinguish, with the unaided eye, the type of net-necrosis under consideration from wilt types of vascular discoloration (*15, Pl. II*), the writers often have found it very difficult and even impossible to do so in slight and, sometimes, in moderate cases. Recourse to microscopic examination then has been necessary, the phloem being the tissue showing necrosis instead of the xylem. As will be described more in detail later, other leafroll characteristics besides necrosis of the phloem appear to be inseparable from net-necrosis. Net-necrosis tubers are often hard and firm (Pl. 4, A), as are leafroll tubers, and bluish on the stem end. Frost-

necrosis of the net type may have a similar appearance, but the writers have not found any frost-necrosis of the ring or blotch types in their net-necrosis material and have found, as reported by Jones and others (*12, p. 36, 45*), that frost-necrosis induced experimentally (Pl. 4, B) was not accompanied by any of the symptoms characteristic of leafroll.

SPINDLING-SPROUT

As with other descriptive names for diseases for which no pathogene has been described, it is quite probable that with "spindling-sprout" more than one cause is involved. The type considered in this paper is shown in Plates 3, B; 4, A, C; and 5, A.

GEOGRAPHICAL DISTRIBUTION

It may be inferred from the reports on the distribution of leafroll that it occurs wherever the Irish potato is grown. Orton (*15*) mentions its occurrence in Austria-Hungary, Denmark, Germany, Holland, Sweden, Switzerland, and the United States. It is also reported to be in France and Java (*17*). Collaborators for the Plant Disease Survey (*11, p. 53*) have noted its occurrence in the States of California, Colorado, Connecticut, Georgia, Idaho, Iowa, Maine, Maryland, Massachusetts, Michigan, Minnesota, Montana, Nebraska, New Jersey, New York, Ohio, Oregon, Pennsylvania, South Carolina, Tennessee, Utah, Vermont, Washington, and Wisconsin. Wortley (*24*) states that he examined more than 60 varieties of potatoes affected by this disease in America—in the East from Florida to Maine; in the Middle West and West in Michigan, Minnesota, and Colorado; in Canada in the Provinces of Ontario, Quebec, New Brunswick, Nova Scotia, and Prince Edward Island; and in Bermuda.

Data are very few concerning the extent to which net-necrosis is found geographically. Reference to Table VI, and to the authorities already quoted in regard to the symptoms shows that it is found generally in Maine and at least to some extent in other States. A form of spindling-sprout may sometimes be a result of net-necrosis, as will be shown later, and so may possibly be found wherever the latter disease appears.

ECONOMIC IMPORTANCE

Estimates made by the collaborators of the Plant Disease Survey (*11, p. 53*) show that in the United States the percentage of plants affected with leafroll may range from 1 to 95. The presence of the disease in almost all the potato-growing sections, including the most important seed-growing centers, produces a very favorable condition for its continued spread. This is more particularly the case where the agents of its dissemination are most abundant, as indicated in the following pages. Leafroll causes considerable losses wherever an appreciable percentage of the plants are affected, because diseased hills produce tubers which may be greatly reduced in size and number. Plants severely diseased will

produce only culls, and these will result in practically a total loss to the grower. The results of studies dealing with the effect upon yield are given in Table I.

TABLE I.—*Effect of leafroll upon yield*

Lot No.	Variety.	Total number of hills.	Percentage of hills leafroll.	Total yield (in pounds).	Yield rate (in pounds per hill).
1919–AF 17, 18, 19	Irish Cobbler	14	0	20	1.43
1919–AF 30 and 31	do	6	100	5	.83
1920–20	Irish Cobbler, strain A	1,195	0	2,052	1.72
1920–24	do	909	10	1,220	1.34
1920–22	Irish Cobbler, strain B	639	0	943	1.47
1920–23	do	616	3.3	722	1.17
1920–28	do	150	100	37	.25
1920–26 and 29	Irish Cobbler	376	100	153	.41
1920–P. I. 13	do	8	100	4.5	.56
1920–P. I. 15	do	12	0	21	1.75

[a] The year of planting is given as part of the lot number.

In Table I the two 1919 lots and lots 1920–P. I. 13 and 15 were hill-selected and planted according to the hill- and tuber-unit method. The others were planted in bulk. As indicated, in two cases a single strain was divided into two or three lots, which may be compared with greater value than lots from different strains. The difference in yield between the two comparable 100 per cent leafroll lots—those planted in bulk in 1920—is due largely to differences between the strains. It is doubtful whether the small amounts of leafroll in lots 1920–24 and 1920–23 are entirely responsible for the reduction in yield rate as compared with the corresponding healthy lots, 1920–20 and 1920–22. However, it is clear that the yield rate of entirely leafroll stock is very materially reduced as compared with that of healthy lots.

When net-necrosis appears as a symptom of leafroll, tubers which otherwise could be used for culinary or exhibition purposes become of less value. Even the usual severe effects of leafroll upon the plant are accentuated by the phloem-necrosis of the tuber, as shown in Plates 5, B, and 6, A. The comparative infrequency of its appearance makes it less important than leafroll, but its relation to leafroll makes the latter, as the fundamental trouble, still more undesirable.

Plate 7, A, B, shows a row, and Plate 7, C, shows a plot of dwarfed plants produced by net-necrosis seed. The effect of net-necrosis upon the yield is considered in Table II. Data concerning the lots grown at Highmoor Farm (of the Maine Agricultural Experiment Station, in southern Maine) and in 1917 at Aroostook Farm (Station Farm in northeastern Maine) were secured, respectively, by Dr. W. J. Morse, plant pathologist, and Mr. G. B. Ramsey, then assistant plant pathologist. These data, in so far as they are concerned with symptoms, are indicated in the table by quotation marks.

TABLE II.—*Effect of net-necrosis upon yield*

Lot No.[a]	Variety.	Sub-lot No.	Place grown.	Condition of seed and plants.	Amount tested for yield.	Yield rate.	Ratio of yield rate.
1916-D	Green Mountain		Highmoor Farm	"Healthy"		325 bushels per acre	100
1916-R	Rural New Yorker		do	do		do	100
1916-G	Green Mountain		do	"Net-necrosis, leafroll" (Pl. 7, A)	93 hills	100 bushels per acre	31
1916-B	do		do	"Net-necrosis leafroll" (Pl. 7, B)	do	38 bushels per acre	12
1917-D	Green Mountain (Davis strain)		do	"Healthy"	300 hills	244 bushels per acre	100
1917-S	Green Mountain	1	do	"Net-necrosis"	247 hills	110 bushels per acre	45
1917-S	do	2	do	"Not net-necrosis"	16 hills	154 bushels per acre	63
1917-A	do	1	do	"Net-necrosis"	231 hills	134 bushels per acre	55
1917-A	do	2	do	"Not net-necrosis"	180 hills	172 bushels per acre	70
1917-D	Green Mountain (Davis strain)		Aroostook Farm	"Healthy"		274 bushels per acre	100
1917-S	Green Mountain	3	do	"Net-necrosis in some. Many leafroll" (Pl. 7, C)	259 hills	84 bushels per acre	31
1917-A	do	3	do	do	169 hills	101 bushels per acre	37
1918-L	Green Mountain (Lowell strain)		Highmoor Farm	"Healthy"	$\frac{1}{160}$ acre	221 bushels per acre	100
1918-R	Green Mountain	1	do	"Severely net-necrosis"	do	36 bushels per acre	16
1918-R	do	2	do	"Slightly net-necrosis"	do	145 bushels per acre	66
1919-HM	do		Aroostook Farm	Healthy	$\frac{1}{8}$ acre	2 pounds per hill	100
1919-MM	do		do	Mosaic	do	1.6 pounds per hill	80
1919-HB	Bliss Triumph		do	Healthy	do	1.4 pounds per hill	70
1919-MB	do		do	Mosaic	do	.9 pound per hill	45
1919-48	Green Mountain (Davis strain)		do	Leafroll	11 hills	.75 pound per hill	38
1919-21	Green Mountain		do	do	17 hills	.73 pounds per hill	37
1919-58	do		do	do	48 hills	.69 pounds per hill	35
1919-66	do		do	do	23 hills	.52 pounds per hill	26
1919-39	do		do	Net-necrosis, leafroll	17 hills	.50 pounds per hill	25
1919-47	do		do	do	8 hills	.64 pounds per hill	32
1919-84	Beauty of Hebron [b]		do	do	40 hills	.36 pounds per hill	18
1919-85	Seedling [b]		do	do	12 hills	.46 pounds per hill	23
1919-116	Up-to-Date [b]		Presque Isle Laboratory plots.	do	do	.38 pounds per hill	19
1919-117	Early Six Weeks		do	do	4 hills	.40 pounds per hill	20
1919-118	Sensation [b]		do	do	do	.63 pounds per hill	32
1919-119	Dearborn's [b]		do	do	do	.60 pounds per hill	30
1919-120	Early Sunrise [b]		do	do	8 hills	.07 pounds per hill	4
1919-121	Early Michigan [b]		do	do	do	.20 pounds per hill	10
1919-123	New White Hebron [b]		do	do	24 hills	.56 pounds per hill	28
1919-124	Green Mountain (Davis strain)		do	do	40 hills	.43 pounds per hill	22
1919-125	Green Mountain (Lowell strain)		do	do	96 hills	.54 pounds per hill	27

[a] The year of planting is given as part of the lot number.

[b] Identification made and seed furnished originally by Prof. William Stuart and Mr. P. M. Lombard, of the Office of Horticultural and Pomological Investigations, Bureau of Plant Industry, United States Department of Agriculture. Source of seed in 1919 was Highmoor Farm.

The comparisons in Table II deal with small lots for the most part, chiefly because of the scarcity of net-necrosis material. They indicate that while leafroll without net-necrosis reduces the yield greatly, both together reduce it still more.

TRANSMISSION OF LEAFROLL

TRANSMISSION BY TUBERS

Transmission of leafroll through the tubers has been noted by different investigators studying this malady (*15*, *17*, *22*). Observations made by the writers upon the same strains of leafroll diseased stock since 1916 show that these stocks consistently have developed plants with the characteristic leafroll symptoms. Tuber production has practically ceased in some of these. Others continue to form plants and tubers as in 1916 but always show leafroll.

TRANSMISSION BY GRAFTING

TUBERS

Since mosaic of Irish potatoes was successfully transmitted by the writers by means of tuber grafts (*20*), similar methods were followed with leafroll. In May, 1919, 20 tubers of approximately the same size and shape were selected from healthy and from leafroll stock. The freshly cut surfaces of a healthy half tuber and a leafroll half tuber were brought into intimate contact and were kept there with rubber bands. Planting followed immediately. The other half of each of the healthy tubers was planted separately as a control. A set of 7 tubers grafted with the halves from healthy tubers served as an additional control.

At the end of the growing season all the control plants were free from leafroll. Three of the 20 healthy half tubers grafted on leafroll half tubers had produced plants with leafroll. Upon examination it was found that these 3 were the only ones which had formed organic union with the corresponding diseased halves. One of the 3 plants thus inoculated through tuber grafting, together with the control, is shown in Plate 8. These results confirm similar ones secured by Quanjer (*17*).

STALKS

In the summer of 1919 a healthy stalk in each of three hills of the Green Mountain variety was grafted with a scion taken from a leafroll plant of the same variety. The stalks at the time of grafting were about 16 cm. tall. At the same time and in a similar manner a stalk in each of four Irish Cobbler hills was grafted. These grafts were in the open field, in three 4-hill tuber units.

By the end of the growing season in the seven different grafted hills each of the shoots growing from the stock just below the graft developed

leafroll. At this time the five ungrafted hills from the same tuber units were still free from leafroll. In the fall of 1919 the tubers from these grafted and ungrafted hills were reserved for planting in 1920. Observations on the second-generation plants in 1920 showed that all the tubers from the leafroll grafted vines produced leafroll plants, while those from four of the five ungrafted hills gave rise to healthy vines. The infection of one control hill was probably due to inoculation by aphids in 1919. These results confirm similar ones secured by Quanjer (*17*) with grafts.

In view of results already obtained with aphids and to be described later, grafts that were made in the field in 1920 were covered with insect cages. Three Irish Cobbler tubers were taken from a lot which was rogued in 1919 and which contained less than 1 per cent of leafroll in 1920. Each was cut into 3 pieces, and each tuber unit was planted under a cloth-covered insect cage. Scions from leafroll plants in the open field were grafted on July 15 upon 4 stalks in each cage, 2 in the second and 2 in the third hill of each tuber unit. All the 12 scions but 1 lived until August 25, when the hills were dug. In spite of precautions, one or more individuals of the potato aphid (*Macrosiphum solanifolii* Ashm.)[1] were introduced into each of the first and second cages, with the result that by August 20 such aphids were numerous. None were found in the third cage at any time. The results were the same for all 3 tuber units, and it seems probable that the aphids had not been abundant long enough to contribute to them. On August 25 each of the 6 hills with grafts, except 1 in the second cage, showed leafroll in the top of a branch coming from a stock just below the graft. One such branch and a control are shown in Plate 6, B, C.

TRANSMISSION BY APHIDS

INDICATIONS OF NATURAL TRANSMISSION FROM FIELD OBSERVATIONS

During 1917 each healthy control lot was planted in the same row with, or in an adjoining row to, leafroll stock of the same strain and variety. Leafroll appeared in 1918 in the progeny of hills that during the previous season apparently were free from leafroll. The healthy hill-selected lots were planted in 1918 and 1919 in a section of the plot more apart than in 1917 from the leafroll lots of the same variety, being from 2 to 12 meters distant. Twenty-five per cent of the 438 hills of control lots, representing Green Mountain, Irish Cobbler, Early Rose, and Burbank varieties, showed leafroll in 1918. Only 13 per cent of the 240 control hills, representing the same varieties, were leafroll in 1919. In 1920 the percentage of leafroll hills, from a total of 336 control hills of the same varieties as in 1919, was 12. It is assumed that, as

[1] Identified by Dr. Edith M. Patch, Entomologist of the Maine Agricultural Experiment Station.

with mosaic (*19*, *20*), new infection appearing early in the season is the result of inoculation late the preceding season. It then follows that the decrease in leafroll in 1919 and 1920 in these control lots apparently resulted from the increase in distance from leafroll lots in 1918 and 1919. Leafroll inoculation, although less in 1918 and 1919 than in 1917, could still be made by aphids dispersing from the diseased lots and from the few diseased hills in the control lots.

In addition to the observations made on the foregoing control lots, which were carefully selected hill and tuber units, some were made on stocks planted in bulk. Here the rows were so arranged that two of them, each containing 300 healthy Irish Cobbler hills, were planted on each side of a row of wholly leafroll stock of the same variety. The entire plot then included 5 rows, with leafroll stock in the middle or third row. This test was begun in the season of 1918, when the aphids were very numerous in northeastern Maine. At the end of the season representative separate lots were selected from the apparently healthy rows before the leafroll stock in the third row was dug. In 1919 these lots were planted in separate rows. Observations now disclosed the presence of leafroll in 1 per cent of the stock which in 1918 was grown in the two outer rows. In the stock grown in the two rows next to the leafroll row, 5 and 11 per cent, respectively, developed leafroll in 1919.

The lot which in 1919 was 11 per cent leafroll was again located next a leafroll row in 1919, and the lot 5 per cent leafroll was in the third row from a diseased row. The leafroll plants were rogued from these before the aphids appeared so that the effect of these insects as carriers as well as the effect of removing diseased plants could be ascertained. Examination in 1920 disclosed leafroll in 5 per cent of the stock grown next to leafroll stock in 1919, and leafroll in ½ per cent of the stock grown three rows from leafroll in 1919. In view of the results obtained with aphids as agents of leafroll transmission, described later, the spread of leafroll to healthy stock grown in the vicinity of leafroll plants can reasonably be attributed to these insects. Whatever may be the cause, increase of proximity clearly increased the spread of leafroll in the field, as has been found by Quanjer (*17*), and by Murphy and Wortley (*13*, *14*), and the variation of the spread from place to place and from season to season, as noted by Murphy and Wortley (*14*), is such as might be expected if aphids were the responsible agents.

FIELD EXPERIMENTS WITH INSECT CAGES

Inasmuch as Quanjer (*17*) and the writers regarded leafroll as being a disease of a type similar to mosaic, and as the writers (*19*, *20*) had secured natural transmission of mosaic by means of aphids, it seemed advisable to attempt transmission with leafroll also. Consequently experiments were begun in the summer of 1919 with special precautions taken against the possibility of soil infection. Seventy Green Mountain tubers were

planted in a small plot with the nearest groups of leafroll plants cultivated with different tools and situated at lower elevations at distances of 10, 15, and 40 meters, respectively. The records indicated that no leafroll plants had been grown in the soil of this plot since 1916 or possibly before, and stocks grown in it during 1917 showed no leafroll in 1918 or 1919. Infection from the soil of the plot thus seems to be impossible. No leafroll was present in the plot during 1919, or in the progeny of another bushel lot of the same strain planted elsewhere,[1] except in the progeny of 2 of the 70 tubers. These were grown in two insect cages as 3-hill tuber units. On July 26, spinach aphids (*Myzus persicae* Sulz.)[2] were transferred to these 2 tuber units, respectively, from two caged potato plants grown in pots, the first having leafroll and the second both leafroll and mosaic. The aphids were of a stock originally kept upon radish plants for a number of generations and proved to be nonvirulent in regard to potato diseases (*19, p. 262–264*). After their introduction to the 2 tuber units, the latter before being dug on September 17 showed the external characteristics of leafroll in the top leaves. In 1920 the tubers from these plants were planted with others from the same plot, each being split and producing a 2-hill tuber unit. The observations made in 1920 are summarized in Table III.

TABLE III.—*Results of inoculation with aphids from leafroll plants in field cages in 1919*

Tuber units in 1919.		Tuber units in 1920.	
Total.	Treatment.	Total.	Percentage of leafroll.
42	Caged. No aphids from leafroll plant	500	0
7	Uncaged. Aphids uncontrolled	84	0
1	Caged. Aphids from leafroll plant	[a] 8	63
1	Caged. Aphids from plant with leafroll and mosaic	[b] 7	86

[a] One tuber, both severely net-necrosis and leafroll. For appearance of a leafroll plant of this lot, see Plate 9, A.
[b] 57 per cent mosaic.

Nineteen of the 70 tuber units in this plot are not considered in Table III, being discarded in 1919 at digging time or their progeny being planted in other fields in 1920 and showing no leafroll. It is clear that the introduction of aphids from leafroll plants caused infection and was the only means of transmission of this disease in this plot.[3] Plate 9 shows plants of the second generation of this stock.

[1] Observed by Dr. W. J. Morse.
[2] Identified by Dr. Edith M. Patch.
[3] After the manuscript for this paper had been submitted for publication, a similar and contemporary but entirely independent experiment was reported as giving similar results (BOTJES, Jan Gerhardus Oortwijn. DE BLADROLZIEKTE VAN DE AARDAPPELPLANT . . . viii, 136 p., 8 pl. Wageningen. 1920. Inaug. Diss. Literatuur, p. 118–122. Summary in German, p. 123–136).

GREENHOUSE EXPERIMENTS WITH INSECT CAGES

During the winter of 1919–20, tubers from healthy Irish Cobbler potato plants were split in two and the halves planted in separate pots in the greenhouse at Washington, D. C. When the plants from these half tubers had reached a height of a few centimeters, some of them were inoculated by means of spinach aphids (*Myzus persicae* Sulz.),[1] which were transferred to the leaves after having been brushed from leafroll plants of the same variety into a wide-mouthed bottle. The aphids were allowed to feed for a few weeks before being killed with tobacco fumes. In each case the control plant from the corresponding half of the same tuber was grown under similar conditions and remained healthy. Additional data regarding these inoculations are given in Table IV.

TABLE IV.—*Leafroll inoculations by means of aphids*

Inoculated plants.								Control plants.
Plant No.	Inoculation.			Insect cage.	Date of fumigation.	Date of first symptom.	Later observations.	
	Date.	Number of aphids.	Height of stalks.					
	1920.		*Cm.*					
332a	Feb. 9	About 50..	3 to 6	Present to Mar. 5.	Mar. 5	Mar. 5	Apr. 2, severe leafroll.	332b, not caged or fed upon by aphids.
340a	Feb. 19	About 100.	5 to 8	Present...	Mar. 10	Mar. 19	.....do.....	340b, like 332b.
350a	Feb. 24	...do.....	4	None.....	Mar. 17	...do....	.....do.....	350b, like 332b.
351a	...do....	...do.....	4	...do.....	...do....	...do....	.....do.....	351b, like 332b.
353b	...do....	...do.....	4	...do.....	...do....	...do....	.....do.....	353a, about 100 aphids from mustard Mar. 13, when 4 cm. high.
352b	Mar. 2	...do.....	4	...do.....	...do....	Mar. 31	Apr. 13, severe leafroll.	352a, like 332b.
355a	...do....	...do.....	4	...do.....	...do....	...do....	.....do.....	355b, like 332b.
359a	...do....	...do.....	4	...do.....	...do....	...do....	.....do.....	359b, like 332b.
.....	.....	.....	.....	.....	.....	.....	.....	356a, like 353a.

From the data presented in Table IV it is apparent that the first symptom was noted from 24 to 29 days after inoculation. This consisted of a slight rolling of the lower leaves. Later this and other characteristic leafroll symptoms became severe, consisting of pronounced rolling and stiffness of the lower leaves, rolling in many cases of the majority of the upper leaves, slight marginal reddish discoloration of many leaves, and rigidity of the whole plant. On half the plants the aphids were killed from 9 to 14 days before the first symptoms appeared and in any case from 23 to 27 days before the symptoms were noted as being severe. Moreover, the control plants which were treated with nonvirulent aphids were still free from leafroll when last observed on April 15, so that aphids alone could not be considered as the cause of the leafroll symptoms.

[1] Identified by Dr. A. C. Baker, Entomologist, Deciduous-Fruit Insect Investigations, Bureau of Entomology, United States Department of Agriculture.

One such control is shown in Plate 10 with the corresponding inoculated plant.

On April 15, 1920, the vines were still green but had made practically full growth. At this time the tubers were harvested from each of the inoculated plants as well as from each of the controls in order to note the amount of infection in the plants of the second generation. In June, about two months later, these tubers were planted in the field. Because of the very brief resting period only a few produced vines of sufficient size to be of value for observation. Two of the second-generation plants, one treated in the first generation with aphids from a leafroll plant and the other its corresponding untreated control, are shown in Plate 11. The former shows very marked leafroll, but the latter remained healthy. Of the controls three out of eight were sufficiently large to show that they were free from leafroll. The remainder had made less than 3 cm. growth when a killing frost ended the season; and hence, although apparently healthy, they were too small for notes to be significant. Of the eight plants whose parents were inoculated, five developed marked leafroll symptoms. The remainder, like the controls, were not yet large enough for notes at the end of the season. Even though not every plant in the second generation made growth adequate for observations, yet the fact that the remainder of the vines disclosed such marked differences, healthy in the case of control stock and distinctly leafroll in the case of inoculated stock, confirms the evidence that the leafroll of the first generation was transmitted by aphids.

GREENHOUSE EXPERIMENTS WITHOUT INSECT CAGES

During the winter of 1919–20 when cage experiments with spinach aphids were being conducted in the Washington greenhouse, another set of healthy potato vines was exposed there to aphids dispersing naturally from leafroll plants. The aphids were of two kinds, mixed, most of them being the potato aphis (*Macrosiphum solanifolii* Ashmead)[1] and the rest the spinach aphis.[1] The healthy potato plants thus exposed to aphid inoculation were located in greenhouse A, as is indicated in Table V. They were grown in 8-inch pots which were within 1.5 meters of the aphid-infested leafroll potato vines of the Irish Cobbler and Green Mountain varieties. Aphid dispersal took place freely during the last week in February, 1920, the healthy plants being half grown. In greenhouse B, as is shown in Table V, a control set of healthy plants similar to those in greenhouse A was set among leafroll plants. The conditions in greenhouse B were like those in greenhouse A with the exception that aphids were eliminated by weekly tobacco fumigation.

On April 13, 1920, when the tubers were harvested, two Irish Cobbler plants of series No. 1 and two Green Mountain plants of series No. 2

[1] Identified by Dr. A. C. Baker, of the Bureau of Entomology, United States Department of Agriculture.

showed leafroll. The tubers of these four plants as well as those of the remainder in both greenhouses, were reserved for study of the second-generation plants. The appearance of this second-generation stock in the experimental field plots is indicated in Table V.

TABLE V.—*Transmission of leafroll by aphids dispersing naturally in the greenhouse*

Series No.	Variety.	First-generation plants.			Second-generation plants.		
		Time of planting.	Location of experiment.	Distance from leafroll plants.	Total number.	Number leaf-roll.	Percentage leaf. roll.
1	Irish Cobbler...	Nov. 28, 1919	Greenhouse A; leaf-roll plants and aphids present.	0.5 to 1 meter.....	16	9	56
2	Green Mountain.	Dec. 3, 1919	.....do.....	0.5 to 1 meter.....	7	3	42
3	.....do.....	Jan. 12, 1920	.....do.....	Next to leafroll plants	8	5	63
4	.....do.....	.....do.....	.....do.....	1.5 meters.....	18	4	22
5	Irish Cobbler...	Jan. 29, 1920	Greenhouse B; leaf-roll plants but no aphids present.	0.5 to 1.5 meters.....	13	0	0
6	Green Mountain.	Dec. 18, 1919	.....do.....	1.5 to 3 meters.....	14	0	0

From Table V it is evident that leafroll developed in a high percentage of the progeny of the healthy potato plants subjected to infestation by aphids from leafroll potato vines. On the other hand, it is equally apparent that all the healthy plants not exposed to aphid infestation remained entirely free from leafroll in both the first and second generations. Furthermore, it is clear that a higher percentage of leafroll developed as the plants of the first generation were closer to leafroll plants. It will also be noted that contact of healthy vines with leafroll foliage was neither sufficient nor essential for transmission of this disease, and that there was no contact of roots. The dispersal of aphids from leafroll foliage to healthy foliage was alone effective in transmitting leafroll.

In the spring of 1920, 120 mostly healthy tubers were split into halves and were put into a warm place and caused to sprout. One-hundred of these were used for other experiments, and four of the 100 produced leaf-roll plants from both halves, as a result of infection of the stock in 1919 or before. The remaining 20 furnished 40 halves. A half from each of the 20 was kept in an individual insect cage for a week together with a leaflet infested, when introduced, with spinach aphids and secured from a diseased potato plant grown in the Maine Agricultural Experiment Station greenhouse at Orono, Me. The aphids varied in number from about 30 to about 100, and at least some of them dispersed to the sprouts, which were from 2 to 5 mm. in length, before being killed by tobacco fumigation. The other half of each tuber was untreated except for similar fumigation. The half tubers were planted without further cutting. Ten halves were fed upon by aphids from mosaic potato foliage; of these,

3 produced mosaic plants as did the corresponding untreated halves, 2 produced healthy plants as did the corresponding untreated halves, 5 produced mosaic plants with the corresponding plants healthy, and none whether treated or not produced a leafroll plant. Ten other halves were fed upon by aphids from leafroll potato foliage; of these, 5 produced leafroll plants while the corresponding halves and the other five pairs of halves produced plants with no leafroll. Thus aphids from leafroll plants inoculated the sprouts of 50 per cent of the half tubers fed upon, while in the control set aphids from mosaic plants inoculated 71 per cent of the half tubers not already diseased. The method used in this experiment is probably not duplicated by natural conditions except for the remote possibility of inoculation at the rhizome scar by aphids reaching and adhering to the tubers at digging time. However, it served to confirm in one generation of plants that which could be proved only by growing the second generation in certain other experiments described in this paper—that is, the ability of aphids to transmit leafroll about as readily as they can transmit mosaic.

TEST OF SOIL TRANSMISSION

In a test regarding the soil harboring of mosaic (*20, Table VIII*), 19 rows of mostly healthy Green Mountain potatoes were planted in 1919 across the location of 14 plots of the preceding year, when 1 plot was of Irish Cobbler potatoes all leafroll and another was of miscellaneous varieties partly leafroll. No leafroll was noted in any of this stock in 1919, or in 1920 except 4 hills in the part of the stock grown on the ground previously planted with miscellaneous varieties. Unless leafroll differs from mosaic by being nontransferable between varieties (*20 p. 324*), these negative results indicate that the danger of leafroll overwintering in the soil is small and is probably due to ungathered diseased tubers which may grow and produce other such tubers and also produce sources for infection by aphids.

Similar negative results in regard to overwintering have been secured by Quanjer (*17*). His explanation of the positive results of proximity on the basis of contamination passing through the soil is, in the opinion of the writers, hardly more plausible than one on the basis of transmission by dispersing aphids. His experiments, as far as can be learned by the writers, were not performed in such a way as to preclude the possibility of inoculation by aphids, and the experience of the writers has shown that with special precautions it is very difficult to control these insects completely and that ordinarily they are abundant. Judging from his figures (*18*), apparent soil transmission was correlated in the first generation with other differences than root contact alone—namely, less complete isolation of the plants by means of pots, more vigorous growth, and a difference in location. All these factors may have influenced the

development and dispersal of virulent aphids.[1] The question of soil transmission will remain open until it is demonstrated with all aerial insects eliminated and even then will be solved more completely when the exact means, possibly either subterranean insects, root contact, soil water, or the soil itself, is disclosed. Wortley (*24*) also reports negative results.

RELATION OF LEAFROLL TO NET-NECROSIS AND SPINDLING-SPROUT

RELIABILITY OF NET-NECROSIS AS AN INDICATOR OF LEAFROLL

A number of separate lots of net-necrosis tubers have been planted in Maine during the past few years, beginning with 1915. The data concerning many of these are given in Table VI. Omission is made of net-necrosis lots which were known to have originated from leafroll hill selection and of those which were grown in the field in 1920. As far as is known, each of the stocks or lots considered in this table is unrelated to the others—that is, no two have had a common origin, though they may have come from the same place. The year of planting is given as part of the lot number. As indicated, sometimes a lot was divided into two or more sublots. The data for all plants grown at Highmoor Farm are taken from records made by Dr. W. J. Morse, and those for the sublots grown in 1917 at Aroostook Farm from records made by Mr. G. B. Ramsey. Such of these as are concerned with symptoms are indicated by quotation marks. The writers are responsible for data for all other lots. In examining this table there should be kept in mind both the difficulty, already discussed in regard to symptoms (p. 48–49), of always ascertaining net-necrosis, or tuber phloem-necrosis, without microscopic examination, and the possibility that plants infected with the leafroll virus may be so reduced in vitality that they lose the usual symptoms of stiffness and pronounced leafrolling. Compare Plate 1, A, B, for the effect of net-necrosis and leafroll in the field, Plate 2, A, and 4, C, for the effect of greater severity of net-necrosis in the greenhouse, and both the two plants of Plate 5, B, and the two pairs of plants of Plate 6, A, for the effect of net-necrosis upon field-grown leafroll hills. The difficulty regarding symptoms would be aggravated by including doubtful cases in a net-necrosis lot, which, when material for study of the disease is rather limited, may seem more desirable than excluding all but marked and severe cases.

The data in Table VI indicate that there is some relation between net-necrosis and leafroll, inasmuch as 26 lots contained both net-necrosis seed and leafroll plants and came from 15 different places and comprised about as many different varieties. The four other lots either contained net-necrosis seed and perhaps leafroll plants (lots 1915–W and

[1] A more recent publication by one of Quanjer's associates (BOTJES, J. G. Oortwijn, OP. CIT., p. 131) also points out the possibility of insect transmission in the experiments in question.

1915–E) or doubtful cases of net-necrosis and no leafroll plants (lots 1919–54 and 1919–96). It will be noted that whenever all the tubers in a lot were unquestionably or severely affected the plants produced by them were all leafroll or spindling or both.

The preliminary studies having indicated that severe net-necrosis was fundamentally a tuber phloem-necrosis and was always associated with leafroll, attempts were made in 1920 to differentiate more exactly macroscopically between net-necrosis and various types of vascular discoloration not associated with leafroll. A tuber was considered as showing at least a medium stage of net-necrosis if a longitudinal section disclosed vascular discoloration exceeding 1 cm. in extent from the rhizome scar, running through several layers, lacking uniformity in degree of discoloration, and with soggy-looking parenchyma near by. It was considered as being in the severe stage if in addition to the foregoing the discoloration extended into the bud-end half or extended beyond the stem-end third with many of the strands black.

During the planting season of 1920, about 8,000 tubers were examined for the presence of net-necrosis and were planted. Of these, 75 were recorded as being in the severe stage, 89 in the medium, and 87 in either the severe or medium stage with no distinction made. Of the 75, all produced leafroll plants except 3 which did not grow above ground. Of the 89, all but 2 produced leafroll plants. One of the 2 exceptions was among the unusual cases showing the disease only in the eye-end half of the tuber. The second was dug July 26 with another net-necrosis hill of the same lot. It was flabby, with the discolored strands hard and brittle and containing fungous hyphae, discolored xylem, and very little discolored phloem, while the tuber that produced a leafroll plant was rigid, with the discolored strands smaller, more numerous, more extensive, lighter in color, not hard, and containing neither apparent fungous hyphae nor discoloration outside the phloem. It evidently had not been possible to distinguish readily at the time of planting between a wilt tuber and a phloem-necrosis tuber. Of the 87, all but 3 produced leafroll plants, 2 of the exceptions producing wilt plants and the third probably being a wilt tuber.

The practical restriction of net-necrosis diagnosis to leafroll potatoes as just described is made more striking by the following facts. Of the 8,000 tubers examined and planted, 1,000 came from about 10 lots which in 1919 were grown so as to be readily exposed to leafroll infection, or were controls to such lots, and furnished 135 of the diseased tubers, while the remaining 7,000 came mostly from lots concerned with the study of mosaic and furnished only 116 diseased tubers. Furthermore, each of the latter group of 116 tubers came from a lot that either was partly leafroll in 1919 or was artificially inoculated in some way with leafroll, or was near—not exceeding 12 meters at the most—to leafroll lots in 1919.

TABLE VI.—*Lots planted for preliminary study of net-necrosis*

Lot No.[a]	Source.	Variety.	Sub-lot No.	Degree of net-necrosis.	Amount planted.	Place of growing.	Appearance of plants.
1915-W	Ellsworth, Me.	Unknown (white)		"Some not bad"	16 pounds	Highmoor Farm	"Few weak hills."
1915-E	Wells Beach, Me.	Unknown (long red)		"Severe"	8 pounds	do	"All small and weak."
1916-G	Gardiner, Me.	Green Mountain		"Some in all"	204 hills	do	"90 per cent weak and spindling throughout the season." Appear like typical leafroll plants in photograph (Pl. 7, A).
1916-B	Foxcroft, Me.	do		do	119 hills	do	"72 per cent like the 90 per cent of the preceding lot" (Pl. 7, B).
1917-S	South Windham, Me.	do	1	"Present"	314 hills	do	"96 per cent weak and spindling."
Do	do	do	2	"Absent"	27 hills	do	"56 per cent weak and spindling."
Do	do	do	3	"Present in some"	340 hills	Aroostook Farm	"Many plants spindling and dwarfed with a tendency to leafroll" (Pl. 7, C).
1917-A	Kennebunkport, Me.	do	1	"Present"	329 hills	Highmoor Farm	"84 per cent weak and spindling."
Do	do	do	2	"Absent"	243 hills	do	"37 per cent weak and spindling."
Do	do	do	3	"Present in some"	200 hills	Aroostook Farm	"Many plants spindling and dwarfed with a tendency to leafroll" (Pl. 7, C).
1918-R	Brunswick, Me.	do	1	"Severe"	112 hills	Highmoor Farm	"Small and spindling."
Do	do	do	2	"Slight"	125 hills	do	"Healthy."
Do	do	do	3	Present	About 40 hills	Aroostook Farm	Many leafroll.
1918-13, 14	Minnesota	Early Ohio		Present in some	Not stated	do	Most leafroll.
1918-15, 16	do	Irish Cobbler		do	do	do	Do.
1918-18, 19	do	Rural New Yorker		do	do	do	Some leafroll.
1919-G	Greene, Me.	Green Mountain		Severe	1 tuber	Orono greenhouse	Leafroll.
1919-5	Aroostook Farm	Bliss Triumph		Medium in 2 per cent of tubers.	872 hills	Aroostook Farm	Leafroll in 9 per cent of the hills.
1919-54	do	Green Mountain		Slight (?) in 23 per cent of tubers.	61 tubers (whole)	do	None leafroll.
1919-84	Aroostook County, Me.	Beauty of Hebron [b]		Medium to severe	20 tuber units	do	All leafroll.
1919-85	Aroostook Farm	(Seedling) [b]		Severe	3 tuber units	do	Do.
1919-96	Presque Isle, Me.	Green Mountain		Slight (?)	2 tubers (whole)	do	None leafroll.
1919-116	Highmoor Farm	Up-to-Date [b]		Severe or decided	4 tuber units	Presque Isle Laboratory plots.	All leafroll.
1919-117	do	Early Six Weeks		do	1 tuber unit	do	Do.
1919-118	do	Sensation [b]		do	do	do	Do.
1919-119	do	Dearborn's [b]		do	do	do	Do.
1919-120	do	Early Sunrise [b]		do	2 tuber units	do	Do.
1919-121	do	Early Michigan [b]		do	do	do	Do.
1919-123	do	New White Hebron [b]		do	6 tuber units	do	Do.
1919-124	do	Davis Green Mountain		do	10 tuber units	do	Do.
1919-125	Bucksport, Me.	Lowell Green Mountain		do	24 tuber units	do	All leafroll; (See Pl. 2, A.)
1919-CF	Aroostook Farm	Green Mountain		Severe in few tubers.	1,600 hills	Aroostook Farm	3 per cent leafroll.

1920–D	East Union, Me	do		Severe	2 tubers	Orono greenhouse	Leafroll.
1920–A	Bath, Me	Spaulding Rose		Marked	do	do	Do.
1920–G	Wells, Me	Green Mountain		Severe	1 tuber	do	Leafroll; very spindling (Pl. 4, C).
1920–V	Nobleboro, Me	Green Mountain (?)		do	3 tubers	do	Leafroll.

[a] The year of planting is given as part of the lot number.

[b] Identification made and seed furnished originally by Prof. William Stuart and Mr. P. M. Lombard, of the Office of Horticultural and Pomological Investigations, Bureau of Plant Industry, United States Department of Agriculture.

POSSIBILITY OF A COMMON CAUSE OF LEAFROLL AND NET-NECROSIS

In the preceding section of this paper data have been submitted showing that in Maine net-necrosis has been consistently accompanied by leafroll. It may be that net-necrosis, a tuber phloem-necrosis, is caused by the same virus[1] as is leafroll or phloem-necrosis. This hypothesis will be tested further by detailed microscopic studies now under way and by various inoculation experiments begun in 1919, but it explains the available facts and explains them at least as well as does the assumption that the two diseases are caused by different viruses.

Experiments indicate that net-necrosis is apparently nonparasitic in nature and transmissible, like mosaic and leafroll. Numerous attempts by the writers and by others in the same laboratories to find one kind of organism associated consistently with the disease have resulted only in failure. The usual origin of discoloration at the rhizome scar indicates that the source of infection comes from the parent plant. The rare cases already noted as found by the writers in which the phloem discoloration was restricted to the eye-end portion suggest that the disease is carried with the juice. Transmissibility by aphids was demonstrated in the field experiment with insect cages, described on page 54. The leafroll plants from which the insects were taken had been produced by net-necrosis tubers. At planting time in 1920, severe net-necrosis was found in one of the eight tubers from the plant given the third treatment described in Table III, while none was found in the 584 control tubers. The fact that this single tuber showed net-necrosis is explained easily by the theory that one virus causes leafroll and, in the proper conditions, net-necrosis as well.

Correlation between the amount of net-necrosis and leafroll in a number of stocks grown in 1920 has been partly indicated on page 60. This correlation is shown further by data upon the 1,000 tubers in the 10 lots that supplied the majority of the net-necrosis tubers, given in Table VII.

TABLE VII.—*Leafroll and net-necrosis in 1920 in lots healthy in 1919*

Lot No.	Total number of tubers.	Tubers producing leaf-roll plants.		Tubers showing net-necrosis at planting time.	Percentage of leafroll tubers showing net-necrosis.
		Number.	Percentage.		
L-31	227	15	7	2	13
L-34	146	0	0	0	.....
L-37	139	2	1	1	50
L-38	120	15	13	9	60
L-39	102	87	85	62	71
352	111	55	50	36	65
354	50	10	20	5	50
355	55	0	0	0	.....
357	38	22	58	15	68
358	40	6	15	5	83

[1] See p. 74, with footnote, for the writers' use of the term "virus."

A close relation between the two diseases is manifested by facts given in Table VII and elsewhere in this paper. Net-necrosis is never present in lots free from leafroll, is never more abundant than leafroll in lots containing both diseases, and is restricted to the tubers transmitting leafroll. This is in contrast with the usual independence of mosaic and leafroll among lots selected as healthy. Consequently there seems to be a closer relation between net-necrosis and leafroll than between mosaic and leafroll. These facts are hard to explain if net-necrosis is considered to be a disease separate from leafroll, but they are easily reconciled with the theory that the leafroll virus may cause net-necrosis under certain conditions.

This theory may seem to be discredited by the following facts. Leafroll hills usually produce small tubers, but net-necrosis often is found in large ones. Also, net-necrosis often disappears entirely or almost so from a stock while the leafroll remains. Both these questions will be considered in the discussion upon the effect of various factors upon the appearance of net-necrosis, where it is shown that large net-necrosis tubers may come from hills that contracted leafroll late in the season and that in some strains net-necrosis may be largely inheritable. Furthermore, the disappearance of net-necrosis in some varieties might well be expected if it is merely a leafroll symptom brought out by certain conditions.

In this connection it may be pointed out that the indication of leafroll by stem-end browning of tubers was considered by Orton (*15, p. 22*) as no longer being reliable. This conclusion is apparently applicable to stem-end browning in general. It might seem to be warranted even in regard to the net-necrosis type when net-necrosis is absent as a leafroll symptom in certain conditions, to be described in the following sections of this paper, and when net-necrosis modifies the usual appearance of leafroll plants, as described on p. 60.

INFLUENCE OF VARIOUS FACTORS UPON THE APPEARANCE OF NET-NECROSIS

VARIETY, TUBER WEIGHT, AND OPPORTUNITY FOR LEAFROLL INFECTION

It may be noted that in the three lots (L-39, 352, and 357) of Table VII that are more than 50 per cent leafroll the percentage of leafroll tubers showing net-necrosis varies only from 65 to 71. Apparently when the number and percentage of leafroll tubers are high, the conditions governing the development of net-necrosis in such tubers may determine the relative amounts of the two diseases within somewhat narrow limits. In order to determine what factors might influence the development of net-necrosis, comparison was made between different parts of each of a number of lots of which most were divided in 1919 first according to the presence or absence of net-necrosis when planted and later according to the presence, absence, or proximity of leafroll hills in the lot or field. These lots were grown in 1919 either at Aroostock Farm or at the Presque Isle Laboratory plots. During that season all net-necrosis tubers planted at the latter place were in one row at the side of the plot. The tubers were examined for net-necrosis in the spring of 1920, and many of them were planted. The results of the various observations are given in Table VIII.

TABLE VIII.—*Relation of leafroll infection to net-necrosis*[a]

1919.								1920.			
Lot No.	Variety.	Place grown.	Sub-lot No.	Leafroll.	Net-necrosis at planting time.	Nearest leafroll hills.	Nearest net-necrosis hills.	Lot No.	Total number of tubers.	Percentage of tubers leafroll.	Percentage of tubers net-necrosis at planting time.
11	Green Mountain	Aroostook Farm	1	All hills (Pl. 6, A)	All tubers (stem-end sets planted).			L-7	47		28
			2	do	All tubers (bud-end sets planted).			L-8	83		20
			3	Absent	Absent	Next or near in row	As for leafroll hills.	L-9	74		3
15	do	do	1	All hills				L-10	22		5
			2	5 per cent removed				L-3	640	0.2	0
			3	5 per cent				L-4	610	4	0.7
110	do	do	1	Absent	Absent (half tubers planted).	Alternating in row (Beauty of Hebron variety).	As for leafroll hills.	L-31	227	7	1
			2	do	Control halves to sublot 1.	Not alternating	do	L-34	146	0	0
126	Green Mountain (Gilbert strain).	Presque Isle Laboratory plots.	1	3 per cent	Absent	Alternating in row (Green Mountain variety, Davis strain.)	do	L-39	102	85	61
			2	3 per cent	do	1 meter (Irish Cobbler variety).	30 meters	L-38	120	13	7
			3	Absent	do	Sublot 1, next section of row.	As for leafroll hills.	L-37	139	2	1
V-126	Rural New Yorker No. 2.	do	1	All hills	do		18 meters	361-A	5		0
			2	4 per cent discarded	do	Discarded 4 per cent[b]	do	[c]V-126		13	
								[d]49-G	5	0	0
V-127	do	do	1	All hills	do		do	360-A	5		0
			2	4 per cent discarded	do	Discarded 4 per cent[b]	do	[c]V-127		1	
								[d]49-H	5	20	0
V-134	Early Michigan	do	1	All hills	All tubers			L-27	7		0
			2	do	Absent		14 meters	365-A	10		0
			3	13 per cent discarded	do	Discarded 13 per cent[b]	do	[c]V-134		40	
								[d]49-H	6	67	67

V-135	Burbank	do	1	All hills	do		do	362	10		10
			2	21 per cent discarded	do	Discarded 21 per cent[b]	do	cV-135		21	
								d_{49}-M	5	40	0
V-136	Sir Walter Raleigh	do	1	All hills	do		do	363	10		10
			2	6 per cent discarded	do	Discarded 6 per cent[b]	do	cV-136		18	
								d_{49}-O	5	20	0
V-138	Green Mountain (Davis strain)	do	1	All hills	All tubers			L-30	64		59
			2	do	Absent		12 meters	351	80		45
			3	Absent	do	Next in row	do	352	110	50	32
V-139	Green Mountain (Lowell strain)	do	1	All hills	All tubers			L-28	27		93
			2	do	Absent		12 meters	353	30		93
			3	Absent	do	Next in row	do	354	50	20	10
			4	do	do	Near in row	do	355	55	0	0
V-142	Dearborn's	do	1	All hills	All tubers			L-25	12		0
			2	do	Absent		11 meters	364	35		0
			3	25 per cent discarded	do	Discarded 25 per cent[b]	do	cV-142		42	
								d_{49}-P	5	60	0
V-143	Up-to-Date	do	1	All hills	All tubers			L-22	17		0
			2	do	Absent		11 meters	365	30		0
			3	30 per cent discarded	do	Discarded 30 per cent[b]	do	cV-143		71	
								d_{49}-Q	5	80	0
V-144	Sensation	do	1	All hills	All tubers			L-24	8		0
			2	do	Absent		11 meters	366	15		0
			3	6 per cent discarded	do	Discarded 6 per cent[b]	do	cV-144		0	
								d_{49}-R	5	0	0
V-148	New White Hebron	do	1	All hills	All tubers			L-29	59		0
			2	do	Absent		8 meters	356	22	100	0
			3	Absent	do	Next in row	do	357	38	58	39
			4	do	do	Near in row	do	358	40	15	13
V-154	Peach Blow	do	1	All hills	do		5 meters	367	43		0
			2	24 per cent discarded	do	Discarded 24 per cent[b]	do	cV-154		17	
								d_{49}-U	5	0	0
V-155	Early Sunrise	do	1	All hills	All tubers			L-26	2		0
			2	Absent	Absent	6 meters	6 meters	cV-155		0	
								d_{49}-V	5	0	0
V-156	Early Six Weeks	do	1	All hills	All tubers			L-23	7		0
			2	do	Absent		6 meters	368	20		0
			3	18 per cent discarded	do	Discarded 18 per cent[b]	do	cV-156		6	
								d_{49}-SS	6	17	17

[a] Absence of an entry indicates that the datum was not available or is obvious. Many sublots leafroll in 1919 were not planted in 1920 because of the repeated demonstration of the inheritability of the disease, assumption being made that all their tubers would produce leafroll plants. It is obvious that when a lot was all diseased the distance to the nearest diseased lot would be zero.

[b] Discarded during the second week of August.

[c] Part planted in bulk without careful examination for net-necrosis.

[d] Part planted in tuber units after examination for net-necrosis.

An examination of Table VIII discloses that net-necrosis was inherited only in three Green Mountain lots (11, V–138, and V–139). That is, the sublot which was selected as showing net-necrosis in 1919 developed as much disease in 1920 as was shown by any other sublot, or more. However, in the majority of such sublots (in lots V–134, V–142, V–143, V–144, V–148, V–155, and V–156) there was no net-necrosis in 1920, although in three lots (V–134, V–148, and V–156) varietal susceptibility was shown by the fact that some tubers were net-necrosis that were produced by plants becoming infected with leafroll in 1919. This difference between lots in the inheritability of net-necrosis, apparently varietal, might, however, seem to be due possibly to a difference in the weights of the tubers harvested. This question is considered, for the sublots just previously mentioned, in Table IX. It is seen in Table IX that by far the greater number of tubers in all lots were small. Therefore the tendency to inherit net-necrosis was varietal rather than due to any tuber weight.

TABLE IX.—*Relation between tuber weight and net-necrosis at the time of planting in sublots selected for net-necrosis a year previously*

Lot No.	Sublot No.	Percentage of net-necrosis.	Number of tubers weighing—						
			2 ounces.	3 ounces.	4 ounces.	5 ounces.	6 ounces.	7 ounces.	8 ounces.
11	1	28	26	14	3	3	1	.....	.....
	2	20	44	25	9	3	1	1	.....
V–134	1	0	4	3	.....	.....	.....	.....	.....
V–138	1	59	25	24	13	1	.....	.....	1
V–139	1	93	16	6	5	.....	.....	.....	.....
V–142	1	0	8	4	.....	.....	.....	.....	.....
V–143	1	0	11	5	1	.....	.....	.....	.....
V–144	1	0	1	4	2	1	.....	.....	.....
V–148	1	0	41	9	9	.....	.....	.....	.....
V–155	1	0	1	1	.....	.....	.....	.....	.....
V–156	1	0	7	.....	.....	.....	.....	.....	.....

The effect of recency of infection is revealed in Table VIII in lot V–148. No net-necrosis was shown by the 1920 generation of the sublots selected in 1919 on account of the presence of net-necrosis or of leafroll, while the second generation of the sublot selected in 1919 as healthy hills next in the row to leafroll hills contained many net-necrosis tubers of which about three-fourths were affected severely (Pl. 12, A). Apparently in this stock and variety, net-necrosis was dependent upon leafroll inoculation that occurred not earlier than during the preceding season. The possibility of the differences between sublots 2, 3, and 4 of lot V–148 being due also to a difference in tuber weight is considered in Table X.

TABLE X.—*Relation between tuber weight, recency of leafroll infection, and net-necrosis at the time of planting, in sublots of lot V–148, New White Hebron variety*

Sub-lot No.	Condition of plants in the previous season.	Number of tubers weighing—										Percentage of net-necrosis.
		1 ounce.	2 ounces.	3 ounces.	4 ounces.	5 ounces.	6 ounces.	7 ounces.	8 ounces.	9 ounces.	10 ounces.	
1	Leafroll, from net-necrosis tubers	41	9	9	.....	.....	.....	.....	.....	.....	.....	0
2	Leafroll	2	13	4	1	1	1	.....	.....	.....	.....	0
3	Healthy, next to leafroll hills in same row	.....	10	8	9	6	2	2	.....	1	.....	40
4	Healthy, near leafroll hills in same row	.....	6	11	10	7	2	1	2	.....	1	13

It is evident from Table X that while the tubers are smaller in the sublots free from net-necrosis, there is sufficient overlapping to indicate that recency of leafroll infection was the dominant factor in this lot. Such recent inoculation was followed by net-necrosis also in lots 11, 110, 126, V–134, V–138, V–139, and V–156, but not in lots V–127, V–135, V–136, V–142, and V–143. The tendency for the tubers to be lighter in weight as leafroll is more pronounced is not surprising in view of the previous discussion of the effects of leafroll.

Lot V–148 also shows the importance of proximity to leafroll hills. The progeny of the sublot from healthy hills next to leafroll hills contained three times as much net-necrosis (in terms of percentage of tubers diseased) in correlation with four times as much leafroll as was shown by the progeny of the sublot from healthy hills near leafroll hills. In addition, both lots were 8 meters from the nearest net-necrosis lot.

The previous discussion has disclosed the dominance of varietal and recent-inoculation factors over the tuber-weight factor in regard to net-necrosis. The question then arises how much correlation may exist between tuber weight and net-necrosis, and incidentally leafroll infection, among the tubers of any sublot coming from hills in uniform conditions of growth and showing a high percentage of net-necrosis. Such data are given in Table XI. Here the smallest tubers are dealt with first, and more tubers in the sublot are included as progressively larger sizes are considered. For example, the first entry represents a sublot with 70 tubers weighing 2 ounces, 39 weighing 3 ounces and bringing the total considered up to 109 as given in the column headed by "2 to 3," 12 weighing 4 ounces, and so on. The classes of heavier tubers contain fewer individuals and if dealt with separately would fluctuate too much—from 0 to 100 per cent—to permit ready perception of the effect of a gradual increase of the average tuber weight upon the percentage of net-necrosis and of leafroll.

TABLE XI.—*Relation between tuber weight, net-necrosis, and leafroll infection at the time of planting* [a]

1919.			1920.																													
			Total number of tubers weighing—										Percentage of net-necrosis tubers weighing—										Percentage of leafroll tubers weighing—									
Lot No.	Sublot No.	Conditions.	2 ounces.	2 to 3 ounces.	2 to 4 ounces.	2 to 5 ounces.	2 to 6 ounces.	2 to 7 ounces.	2 to 8 ounces.	2 to 9 ounces.	2 to 10 ounces.	2 to 11 ounces.	2 ounces.	2 to 3 ounces.	2 to 4 ounces.	2 to 5 ounces.	2 to 6 ounces.	2 to 7 ounces.	2 to 8 ounces.	2 to 9 ounces.	2 to 10 ounces.	2 to 11 ounces.	2 ounces.	2 to 3 ounces.	2 to 4 ounces.	2 to 5 ounces.	2 to 6 ounces.	2 to 7 ounces.	2 to 8 ounces.	2 to 9 ounces.	2 to 10 ounces.	2 to 11 ounces.
11	1, 2	Net-necrosis and leafroll...	70	109	121	127	129	130	....	....	....	....	27	24	25	24	23	23	....	....	....	....	....	....	....	....	....	....	....	....	....	....
126	1	3 per cent leafroll, between net-necrosis leafroll hills.	14	37	58	71	84	91	99	101	102	....	43	46	53	55	58	58	59	60	61	....	79	78	81	83	86	85	85	85	85	....
V-138	1	Net-necrosis and leafroll...	25	49	62	63	63	63	64	....	....	....	68	65	62	60	60	60	59	....	....	....	....	....	....	....	....	....	....	....	....	....
V-139	1	.....do.....	16	22	27	....	....	....	....	....	....	....	94	95	93	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
V-148	3	Healthy next to leafroll hills in same row.	10	18	27	33	35	37	37	37	37	38	20	22	33	39	43	41	41	41	41	39	30	33	44	55	57	57	57	57	57	58
V-148	4	Healthy near leafroll hills in same row.	6	17	27	34	36	37	39	39	40	....	0	12	11	15	14	14	13	13	13	....	0	12	11	18	17	16	15	15	15	....

[a] Leafroll infection in tubers was ascertained from the appearance of their plant progeny in the early part of the season.

In the 1920 generation of the three sublots that showed net-necrosis in 1919—that is, the first, third, and fourth as given in Table XI—the inclusion of progressively larger sets of tubers tends to reduce the percentage of net-necrosis. Thus, inherited net-necrosis is less common as the average tuber weight is greater. In the 1920 generation of the other three sublots a progressive increase in tuber weight is accompanied by a rather marked increase in the percentage of both leafroll and net-necrosis until 5 or 6 ounces are reached, except in sublot 1 of lot 126, where the increase of tuber weight has more effect on net-necrosis percentage than on leafroll percentage. Here, in the sublots with initial net-necrosis, the fundamental relation is one between leafroll infection and tuber weight, with its exact cause unknown. Initial net-necrosis shows a similar relation to tuber weight because it is proportional to leafroll infection.

It has been pointed out that inheritability of net-necrosis tends to be varietal. Inherited net-necrosis may tend to be more common as the tubers are lighter in weight. Recency of leafroll infection may determine the development of net-necrosis within a given variety. Finally, net-necrosis resulting from recent leafroll infection may tend in a given strain to be more common as the proximity to leafroll hills is greater and to be less common in the lighter-weight tubers, both usually in correlation with a similar tendency shown by the leafroll infection itself.

It may also be pointed out that there was a great difference in leafroll infection, as shown in Table VIII, between the first sublot of lot 126 and that of lot 110. This was not necessarily due to the facts that in lot 126 the healthy hills were of the same variety (Green Mountain) as the diseased hills alternating with them and that in lot 110 they were of the Green Mountain variety, while the diseased ones were not. The latter lot, showing much less leafroll (and net-necrosis) in 1920, was in a field in 1919 in which aphids were later in appearance and fewer in numbers than they were in the plot in which the former lot was grown. Also, it was over 150 meters from the weedy border of the field, while the former lot was about 25 meters from one end of an acre plot with a permanent weed patch at both ends and along one side and so was much nearer to the probable sources of newly hatched aphids. The most important difference seems to have been that of abundance of aphids, which have been shown to be capable of transmitting leafroll.

TEMPERATURE

Jones and others (*12*) have demonstrated that a type of net-necrosis may be induced by certain conditions of low temperature, but have distinguished (*12*, *p. 22*) between frost-necrosis of the net type and inheritable net-necrosis. The same distinction is made by Coons (*7*, *p. 37*). One of the writers at Orono followed the methods of Jones and his coworkers (*12*), except that outdoor conditions were used, and induced

TABLE XII.—*Presence of net-necrosis in unchilled tubers*

Lot No.[a]	Sub lot No.	Place grown.	Variety.	Condition of seed and plants.	Date examined.	Number of tubers.	Percentage of net-necrosis.
1916-G		Highmoor Farm	Green Mountain	"Net-necrosis, mostly leafroll"	Jan. 4, 1917	148	11
1916-B		do	do	do	do	127	2
1917-S	1	do	do	"Net-necrosis"	Feb. 15, 1918, or before	120	23
1917-A	1	do	do	do	do	112	19
1917-A	2	do	do	"Grown near lot 1917-A, sublot 1"	Feb. 15, 1918	80	20
1917-D		do	Green Mountain (Davis strain)	"Healthy, next to lots 1917-S, sublot 1, and 1917-A, sublot 1."	do	100	13
1917-AF3		Aroostook Farm	Green Mountain	No notes on leafroll. In same plot with scattered leafroll lots.	Feb., 1918	15	13 (severe).
1918-S and 1918-A.	2	do	do	Net-necrosis, 65 per cent leafroll	Sept. 15, 1918 (harvest)	100	1
	1				Dec. 7, 1918		49
					Feb. 7, 1919		66
					Apr. 5, 1919		70
1918-S	4	do	do	45 per cent leafroll	Sept. 15, 1918 (harvest)	50	0
					Dec. 7, 1918		22
					Feb. 7, 1919		36
					Apr. 5, 1919		40
1918-A	4	do	do	63 per cent leafroll	Sept. 15, 1918 (harvest)	50	6
					Dec. 7, 1918		8
					Feb. 7, 1919		16
					Apr. 5, 1919		20
1918-R	1	Highmoor Farm	do	Severe net-necrosis, some leafroll	Feb. 22, 1919	71	7
1918-R	2	do	do	Healthy, next to lot 1918-R, sublot 1	do	127	11
1919-5		Aroostook Farm	Bliss Triumph	Leafroll; harvested Aug. 7	Jan., 1920	50	2
1919-11		do	Green Mountain	Net-necrosis, leafroll; harvested Aug. 7	do	30	7
1919-125		do	Green Mountain (Lowell strain)	Net-necrosis, leafroll; harvested Sept. 11	do	271	13 (4 severe).
1919-84		do	Beauty of Hebron	Net-necrosis, leafroll; harvested Aug. 30	do	110	5

[a] The year of planting is given as part of the lot number.

frost-necrosis of the net (Pl. 4, B), ring, and blotch types. Neither of the last two types of injury has been seen in connection with the leafroll type of net-necrosis, which has developed in connection with leafroll tuber transmission under normal conditions of growing, harvesting, and storing, while at the same time thousands of other tubers, healthy in regard to leafroll, were in the same conditions but remained free from net-necrosis. Moreover, a number of lots of tubers have been selected from stocks grown at Aroostook Farm and Highmoor Farm for the study of net-necrosis or leafroll and have been kept in well-heated buildings, so that it was known that there was no chance for chilling after harvesting. These are described in Table XII. Notes upon the first seven sublots were taken by either Dr. W. J. Morse or Mr. G. B. Ramsey. The sources of many are described in Table VI.

It is seen from Table XII that net-necrosis may develop without any exposure to chilling temperatures. Furthermore, a certain test indicates that there is no relation between higher temperatures of storage and the prevalence of net-necrosis. This test consisted of dividing the tubers from lot 1919–125 of Table XII into eight parts so that the range and average of the tuber weight were as nearly alike as possible for the various sublots. Four sublots were kept in constant temperature chambers at 30°, 25°, 15°, and 8° C., respectively. The other four were kept where the temperature fluctuated daily, the daily means averaging 25°, 21°, 15°, and 10°. This was done for about two months, from November 1 to January 7. At the end of this period examination of the tubers disclosed no differences between the various sublots in regard to net-necrosis that were correlated with differences in temperature.

PHYSIOLOGICAL CHANGES

It may be noted that three lots considered in Table XII were examined at the time of harvesting and at bimonthly intervals afterwards. The percentage of net-necrosis increased markedly up to about the middle of February. Cook (*6, p. 26*) reports that this discoloration may be absent at the time of digging but may develop in storage. Apparently changes that occur during the dormant period of the tubers may be involved with the development of the discoloration.

During preliminary examination of net-necrosis tubers in comparison with healthy tubers, it was found that blackening appeared more quickly in cut or crushed tissues of the former tubers than in those of the latter and appeared more quickly than is possible for bacterial growth. The blackening substance was present in uninjured tissues only in the phloem of those with net-necrosis. It affected protoplasm and protein granules but not starch grains. It did not pass through the walls of vessels in the xylem of healthy tuber segments which were allowed to absorb some of the discolored juice. It developed most rapidly in crushed tissues exposed to the air and was characterized by being associated with acid

coloration in litmus solution when from net-necrosis tubers but not when from healthy tubers. Possibly the discoloration of net-necrosis is due to an accentuation of a normal tendency of the potato tuber tissues to darken upon exposure to air. Darkening has been attributed by Bartholomew (*4*) in the case of blackheart to melanin or humin formed by the interaction of tyrosinase and tyrosin in the presence of oxygen. It may possibly be due, in net-necrosis, to identical or similar physiological changes resulting from, or productive of, leafroll infection. This is indicated by the finding of tyrosinase in greater quantities in leafroll tubers (*8*).

RELATION OF NET-NECROSIS TO SPINDLING-SPROUT

In the preceding sections of this paper evidence has been submitted indicating that net-necrosis of one kind is a symptom of leafroll infection. It may be a cause of spindling-sprout, and the latter may thus become a symptom of leafroll. Spindling-sprout was distinctively pronounced in the net-necrosis half of the tuber shown in Plate 5, A. It was consistently characteristic of the net-necrosis tubers selected from lot V–148, sublot 3, of Table VIII, half of which sublot is shown in Plate 12, A, and was accompanied by a reduction in the number of lengthened sprouts. Such reduction in length is also apparent to some extent in sprouts of the companion sublot 2 from leafroll hills, but they show no decided spindliness (Pl. 12, B). Plate 3, B, depicts the appearance of representative tubers from the two groups shown in Plate 12, A. Plate 4, A, illustrates the accompaniment of net-necrosis and another leafroll symptom—namely, maintained rigidity of the tuber—by spindling-sprout in diseased tubers kept with healthy ones in an uncooled room from June 1 to August 11. The type of spindling-sprout caused presumably by net-necrosis has been described also by Stewart and Sirrine (*23, p. 140–141*) and other types with apparently different causes by the same writers (*23*), by Coons (*7, p. 29*), and by Haskell (*10, Pl. 13, Fig. B*).

RELATION OF LEAFROLL TO MOSAIC

As indicated previously, Quanjer considers that both mosaic and leafroll are virus diseases. The writers are of the same opinion. That is, they consider both to be transmissible or infectious diseases for which no pathogene or other cause has been isolated or synthesized.[1]

Regardless of such similarity between the two diseases, it appears to the writers unwise yet to regard the behavior of one as indicating exactly what that of the other will be, at least in commercial fields. Each variety of potato commonly grown in Maine apparently differs in susceptibility both in regard to the two diseases and in comparison with the other

[1] Such isolation or synthesis apparently will be necessary before it will be decided whether the convenient term "virus" represents an ultramicroscopic organism or an enzym. The two sides of the controversy are represented by Allard (*1, 2*) and Chapman (*5*).

varieties respecting either disease. The Irish Cobbler is the only one which in commercial fields often shows more than a trace of leafroll in northeastern Maine, and it seldom shows more than a trace of mosaic, while the Green Mountain and Bliss Triumph varieties are seldom found with only a trace of mosaic.

RELATION OF LEAFROLL TO CLIMATE

The Bliss Triumph variety contracts leafroll readily on Long Island and in Bermuda, in contrast to its behavior in northeastern Maine (*24*). This and similar differences between other geographical regions in regard to the rapidity of the spread of leafroll have been attributed to climatic differences. The question also arises whether or not greater prevalence of sources of infection, the importance of which is suggested by Murphy and Wortley (*14*), may be the chief or only cause of more rapid increase of leafroll in some regions within any given variety. As far as is now known, the greater prevalence of sources of infection on Long Island or Bermuda may now be the more important factor in the greater spread of leafroll there, with greater abundance of aphids helping both now and in the past to increase sources of infection. Climate, in turn, may differ enough to influence the abundance of aphids. In Virginia they are abundant in the winter (*21*) when entirely absent in Maine, except in the egg stage; and it seems probable that in sections intermediate as to climate their season begins earlier than in Maine and that they have greater opportunity to become numerous upon potatoes and to spread leafroll.

Whether or not the relative abundance of aphids is an important factor can be tested best by experiments in which parts of the same stock are grown at two or more places with surrounding conditions similar in regard to leafroll. The prevalence-of-source explanation is not necessarily supported by the less rapid spread of leafroll in Maine, as compared with that of mosaic, from diseased plants to those near by. It is possible that more severe inoculation by aphids is required to transmit leafroll than to transmit mosaic, and that these insects are abundant enough on Long Island and Bermuda to spread both diseases readily, but usually not in Maine. Inoculation of bruised leaves with virulent juice has proved far easier with mosaic than with leafroll. Aphids when introduced artificially have transmitted leafroll within the Green Mountain variety about as readily as they have mosaic (p. 59 and Table III). On the other hand, natural field inoculation by aphids in northeastern Maine evidently would be greater, even for mosaic, if there were more aphids. Hill selections made for freedom from mosaic in partly diseased fields in the valley of the St. John River in Maine ordinarily have given more satisfactory results than those made in the same manner in fields near Presque Isle, in correlation with less prevalence of aphids as indicated by counts made on leaves taken at random at the same time of year. Also, the number of aphids in Maine ordinarily is small enough for interseasonal

variation to have a marked influence upon the spread of mosaic (*19, p. 331*).

Reduction of the spread of leafroll by shade, in comparison with the spread in drouth conditions, has been reported by Wortley (*24, p. 519*). It seems possible that aphids might be more restless and inclined to disperse from plant to plant in drouth conditions and that their dispersal might be reduced in shade conditions by a fungous disease. Such a disease has been noted in Maine by the writers as being more prevalent in insect cages than in the open field during sunny weather and as practically eradicating an abundant infestation within a few days of cloudy weather. It may be suggested that the normal weather conditions of northeastern Maine specially favor any fungous disease of aphids, even as they favor the growth of the potato plant and the development of its important malady, the late blight (*Phytophthora infestans* de By).

CONTROL OF LEAFROLL

Roguing tests were begun in 1919 in order to ascertain whether or not it was possible to free infected potato stock from leafroll plants. Two stocks, lots 20 and 21 of Table XIII, have been grown upon the experimental plots in northeastern Maine since 1916. They were at first a single healthy stock which did not yet contain any leafroll plants in 1918 when it was divided and located in two rows next to a 100 per cent leafroll row. It was exposed to heavy aphid infestation in 1918, and in 1919 it developed leafroll in 5 per cent of the plants from one row and in 11 per cent of those from the other. In 1919 a part (sublot 1) of each of these 1-row lots was rogued, while another part (sublot 2) was not rogued. That season these lots were again located, as indicated, near or next to wholly leafroll stock, and numerous aphids, though not so many as in 1918, appeared during the latter part of the season. At harvesting time only tubers of approximately 4 ounces and more were selected.

Lot 22 as noted was taken from a commercial field having 14 per cent leafroll infection. This field is located fully half a mile from the experimental plots on which lots 20 and 21 were grown. It happened that aphids were very scarce there, only a few individuals to a plant being noted late in the season. Part of this stock was rogued and designated as sublot 1, while sublot 2 of this same stock was not rogued. The selection of tubers was the same as for lots 20 and 21. Observations on these lots are reported in Table XIII.

In view of the varying conditions obtaining in connection with the different lots described in Table XIII, it will be impossible to consider that a single factor caused the differences in leafroll percentages in 1920. Apparently selecting tubers in 1919 reduced the leafroll percentage in the unrogued sublots of lots 1919–21 and 1919–22, while such an effect was prevented in lot 1919–20 by greater proximity to a leafroll row. In sublot 1 of lot 1919–20 roguing prevented an increase in leafroll per-

centage which resulted in sublot 2, in spite of tuber selection, from proximity to a source of virulent aphids. In sublot 1 of lot 1919–21 roguing produced a greater reduction in leafroll than in sublot 1 of lot 1919–20, apparently because of a greater distance from entirely leafroll stock.

TABLE XIII.—*Effect of roguing upon percentage of leafroll*

Lot No.[a]	Variety.	First season.				Second season.	
		Location.	Percentage of leafroll.	Sublot No.	Roguings.	Total number of hills.	Percentage of hills leafroll.
1918–4	Bliss Triumph.	Aroostook Farm plant-disease plot, two rows.	(b)	1	1	1,332	2
				2	0	1,615	8
1919–15	Green Mountain.	Aroostook Farm plant-disease plot, one row.	5	1	1	1,641	0.2
				2	0	1,489	4
1919–20	Irish Cobbler.	Aroostook Farm next all-leafroll row.	5	1	2	1,254	4.7
				2	0	954	10
1919–21	...do.....	Aroostook Farm third row from all-leafroll row.	11	1	2	1,321	.5
				2	0	954	3.8
1919–22	...do.....	Commercial field.....	14	1	1	639	0
				2	0	637	8

[a] The year of planting (first season) is given as part of the lot number.
[b] Rogued so late (August 16) that hot weather and age caused many undoubtedly healthy hills to look as if possibly leafroll.

It is noteworthy that in lots 15 and 22 leafroll was entirely eliminated from sublot 1 by a single roguing. The marked reduction of leafroll in sublot 2 of lot 22 apparently was due to a large percentage of the leafroll vines producing tubers weighing less than 2 ounces and to the selection of large tubers together with the scarcity of aphids in this field. Hence it appears that leafroll may be eliminated from infected stock by roguing, provided that such stock is not in close proximity to leafroll vines infested with aphids, and may be reduced by discarding small tubers. This means that in northeastern Maine leafroll may be controlled more readily than mosaic of potato (*19, p. 270*). Whether this is due to less prevalence of diseased plants or to less susceptibility under the existing conditions of aphid infestation remains to be determined by further investigation. In either case it would not be advisable to expect similar results elsewhere where either diseased plants or aphids are more numerous.

From the data on hill selections (p. 53) it is apparent that this method of selection does not necessarily insure stock free from leafroll if such selection is practiced near leafroll plants infested with aphids. Hence, in view of the fact that elimination of leafroll hills from the seed plot by roguing appears to be fully as effective as hill selection, the former method of eradication seems the more desirable one. From the results on aphid transmission it is very evident that the earlier the diseased plants are

removed from the seed plot the less chance there will be for infection from such plants. This is more particularly the case in those sections of the country where aphids become very numerous relatively late in the development of the vines. Furthermore, isolation of the seed plot from diseased plants in adjoining plots or fields also will contribute to the effectiveness of roguing.

SUMMARY

(1) Potato leafroll of the apparently nonparasitic transmissible type, also called phloem-necrosis, is probably as widely distributed over the earth as are potatoes. Its exact geographical distribution is difficult to ascertain, however, because of the present impossibility of easily identifying it by the isolation of a pathogene or other cause and because of the resulting tendency to apply the name to unrelated diseases with somewhat similar symptoms. The same may be said of net-necrosis and spindling-sprout.

(2) The chief and perhaps the only manner of transmission from season to season in the soil or elsewhere is by means of tubers from diseased plants.

(3) Leafroll usually reduces the yield considerably, and, inasmuch as it is inheritable, it decreases the value of the tubers for seed. It may also lessen the value of the tubers for other purposes.

(4) Leafroll is transmissible from one plant to another by means of grafting either tubers or stalks and by means of aphids.

(5) Net-necrosis is apparently a leafroll symptom, being a discoloration which results from tuber phloem-necrosis and which appears more often as conditions of variety, recency of infection, and weight of tuber are more favorable. It develops in the dormant tubers without relation to differences in the storage temperature. When it occurs as a symptom of leafroll, the effects of the latter are still more detrimental, one being a decided spindliness of the sprouts. Transmission and control of leafroll are thus concerned indirectly with net-necrosis and spindling-sprout.

(6) Leafroll and mosaic are somewhat similar types of diseases.

(7) Inter-regional differences in the spread of leafroll may depend upon differences in climate and in the abundance of aphids.

(8) Roguing has proved to be much more effective in eliminating leafroll than it has been for mosaic, at least in northeastern Maine.

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PLATE 1

A.—Green Mountain potato plant, produced by a net-necrosis tuber and showing leafroll. For healthy plant in the same hill lot, produced by a healthy sister tuber see B.

B.—Healthy control to leafroll plant shown in A.

A

B

A

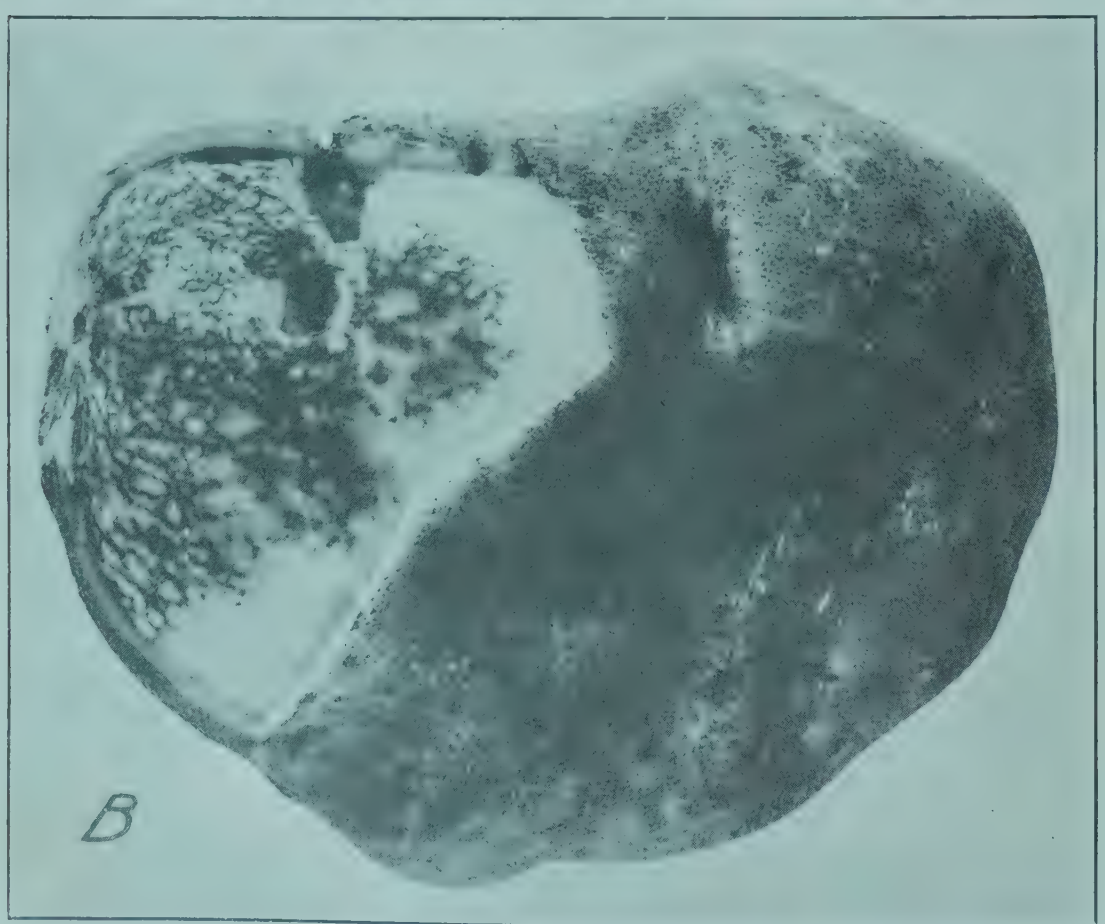

B

PLATE 2

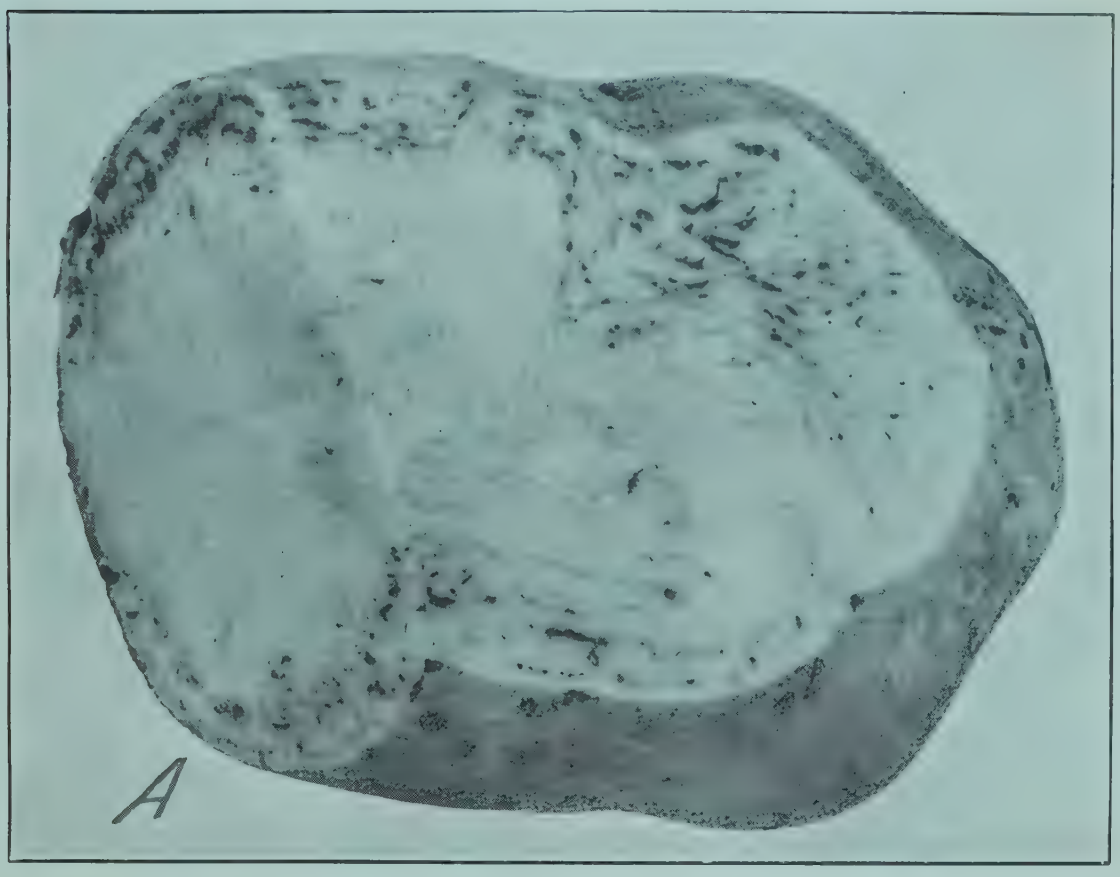
A.—Greenhouse-grown progeny of a net-necrosis tuber from Green Mountain stock (lot 1919–125 of Table VI) showing net-necrosis and leafroll the preceding season.

B.—Tuber with part of stem end cut away to show net-necrosis. (From lot 1920–V of Table VI). The two deep holes are due to elimination of the effect of insect injury.

PLATE 3

A.—Net-necrosis tuber with part of stem end and side sliced off.

B.—New White Hebron potatoes from the same lot, representative of healthy and net-necrosis tubers of this variety. Note spindliness and scarcity of sprouts from the net-necrosis tuber. (See Pl. 12, A.)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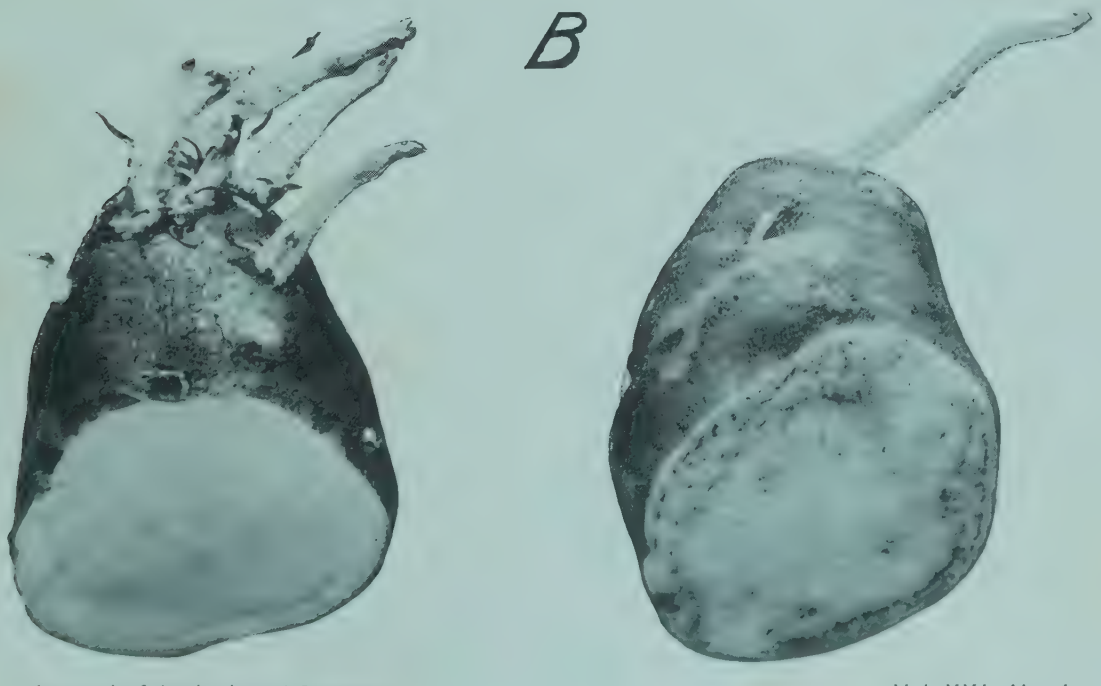
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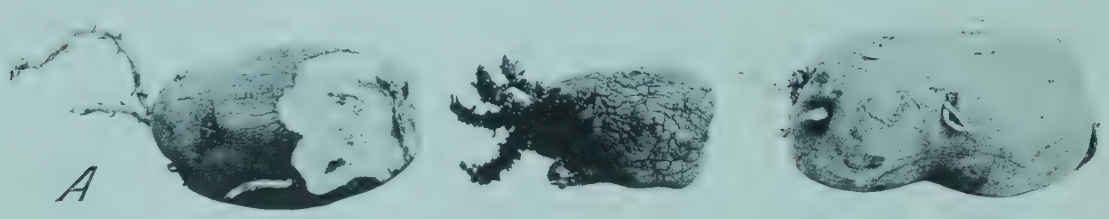
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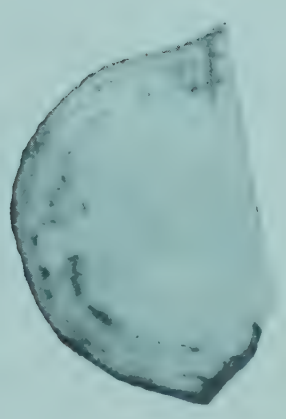
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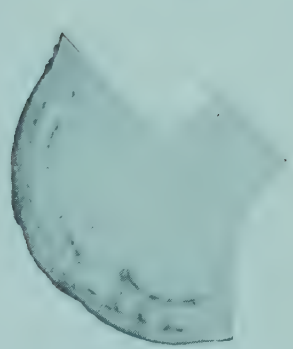


C

PLATE 4

A.—Green Mountain tubers kept in the same sack in a warm room from June 1 to August 11, 1919. The middle one had no net-necrosis and is withered, soft, and bearing normal sprouts. The others had net-necrosis and are smooth, firm, and bearing spindling sprouts.

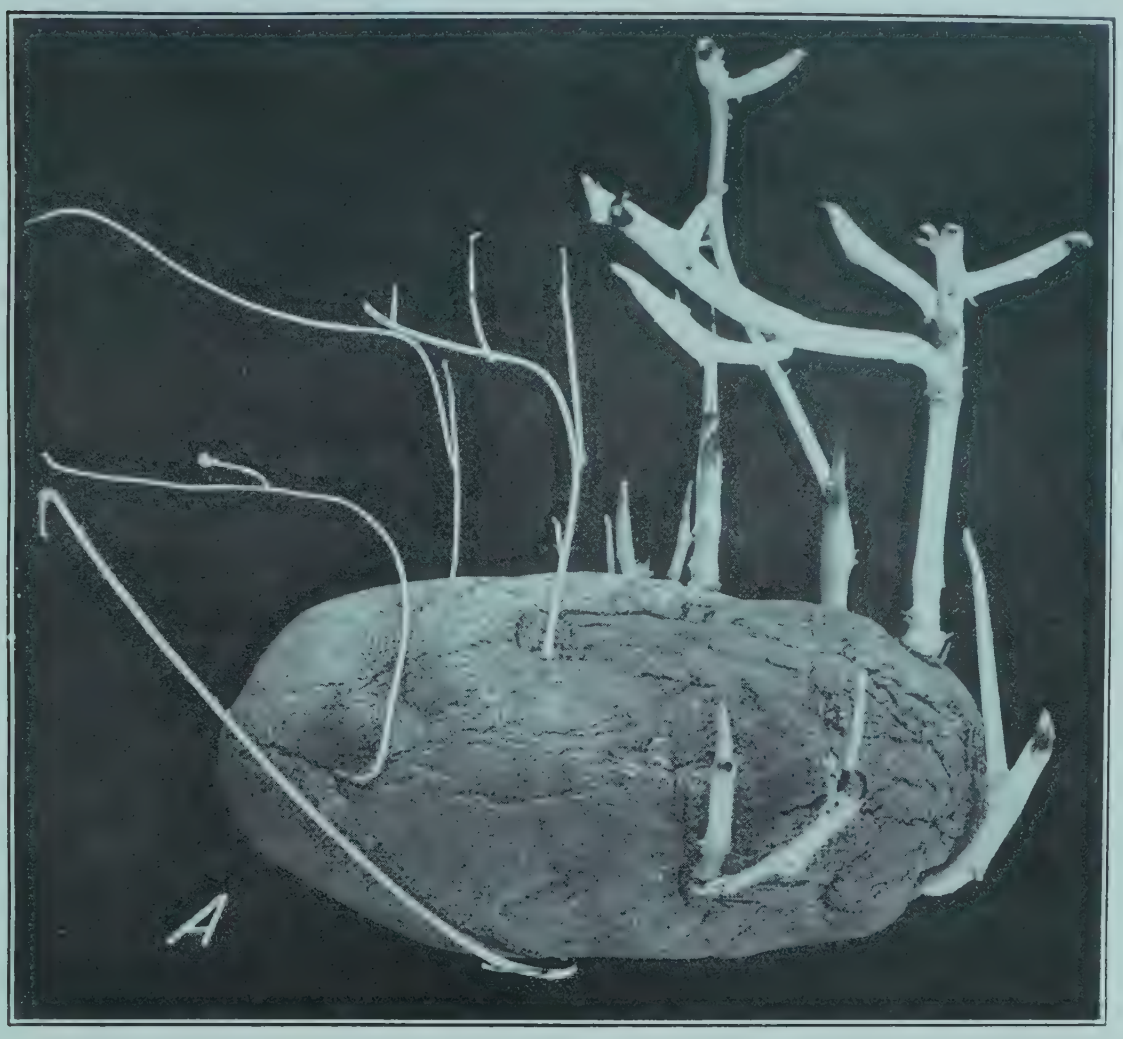
B.—Frost-necrosis of the net type produced by measured exposure to low temperature. Thin slices of Green Mountain tuber cut off to increase the visibility of the injury.

C.—Greenhouse-grown plant produced by a net-necrosis tuber (lot 1920–G of Table VI) and showing spindliness and slight leafroll.

PLATE 5

A.—Tuber with spindling sprouts coming from the part which later was found, by cutting, to be affected with net-necrosis.

B.—Two leafroll Green Mountain hills in the same hill lot, the one on the left produced by a half of a net-necrosis tuber and the one on the right produced by a half of a sister tuber that transmitted leafroll but showed no net-necrosis.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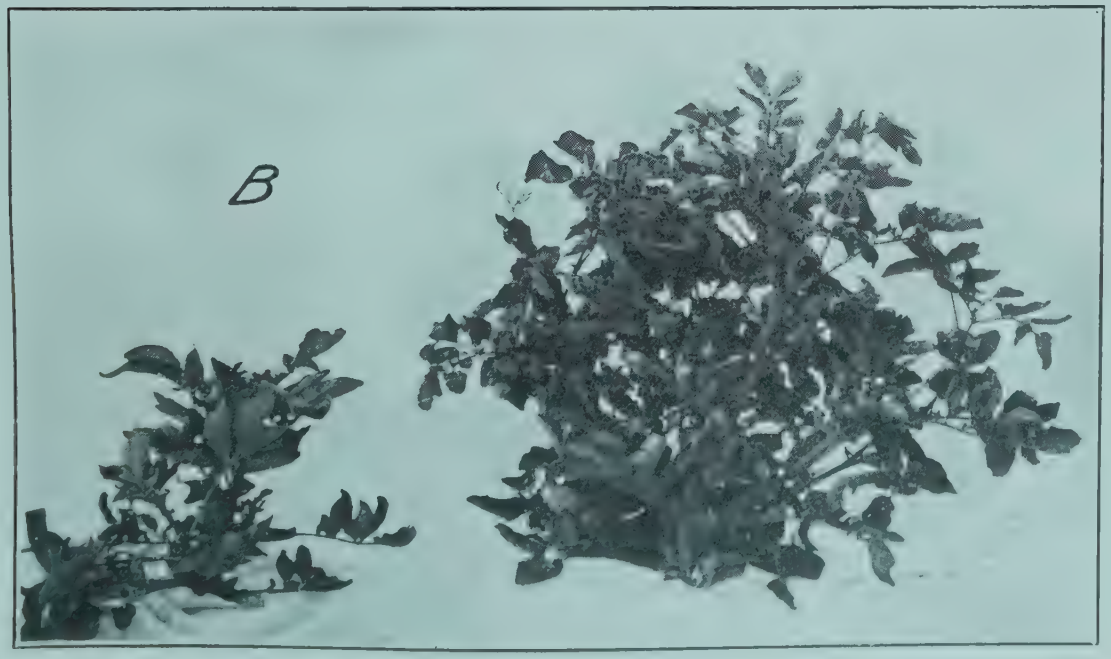
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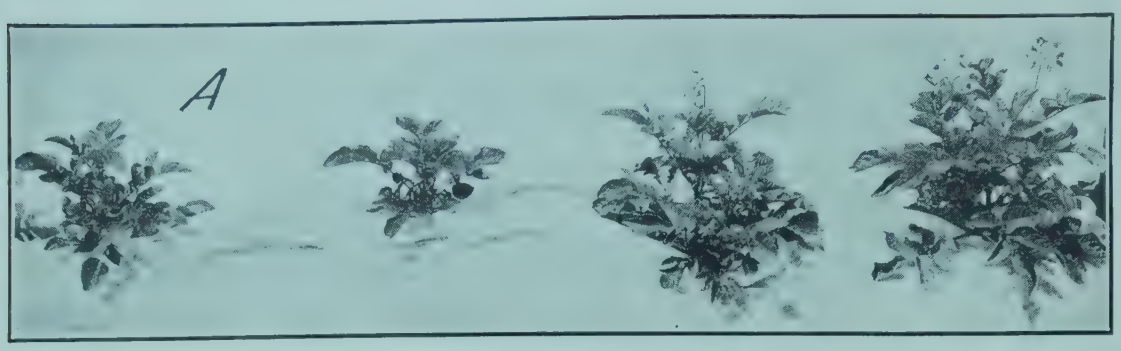
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B

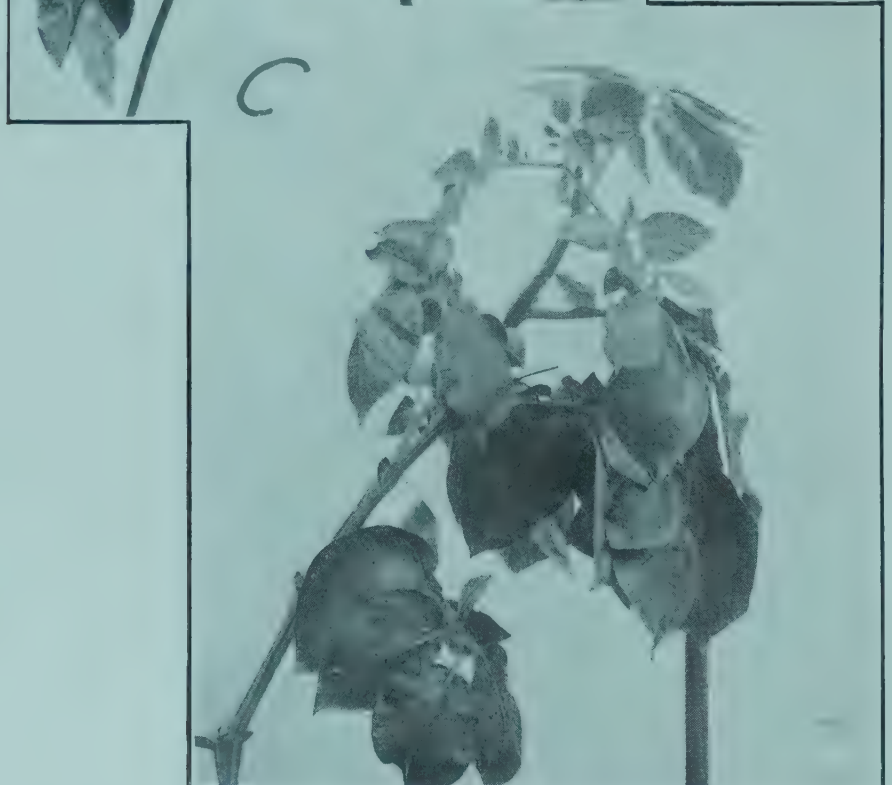
C

PLATE 6

A.—Leafroll tuber unit, Green Mountain variety, with net-necrosis stem-end quarters planted at the left. Photographed July 9, 1919, in northeastern Maine. (Lot 11 of Table VIII.) Note the smaller size and less advanced stage of the plants from the quarters affected with net-necrosis.

B.—Irish Cobbler branch with leafroll in top, from bud of originally healthy stock near leafroll scion. For control grown in the same hill but ungrafted, see C.

C.—Branch from ungrafted stalk. Control in same hill as the branch shown in B.

PLATE 7

A.—Net-necrosis Green Mountain stock (1916–G of Tables II and VI), photographed August 4, 1916, in southern Maine.

B.—Net-necrosis Green Mountain stock (1916–B of Tables II and VI), photographed August 4, 1916, in southern Maine.

C.—Net-necrosis Green Mountain stock (lot 1917–S, sublot 3, or 1917–A, sublot 3, of Table VI), in the foreground and healthy stock of different origin in the background, both grown under the same conditions. Photographed June 27, 1917, in northeastern Maine, when the season was not yet advanced enough for leafroll to be most marked.

A
B
C

A

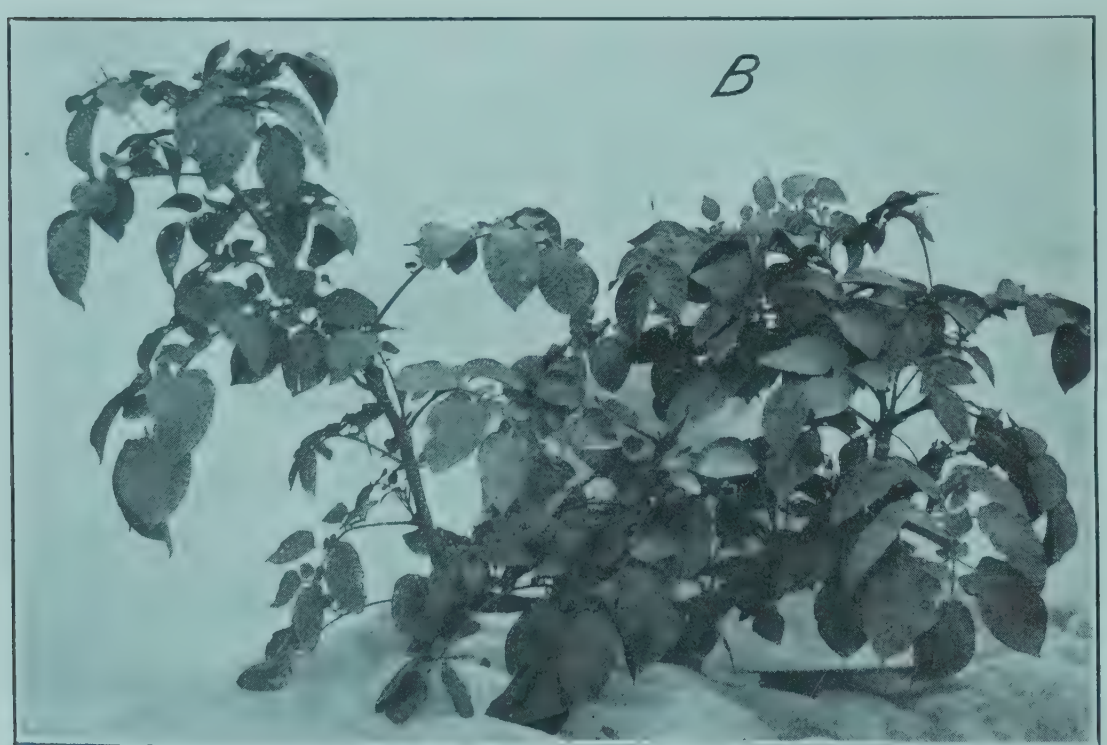
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PLATE 8

A.—Leafroll stalks from Irish Cobbler half tuber healthy when grafted upon leafroll half tuber. Removed from field to secure better photographic facilities and to ascertain absence of parasitic injury. For control, grown from other half of the same tuber, see B.

B.—Healthy control to plant shown in A.

PLATE 9

A.—Second-generation Green Mountain plant, leafroll as result of aphid inoculation of first generation in field insect cages. (See Table III.) For healthy control from the same stock, see B.

B.—Healthy control to plant shown in A.

A

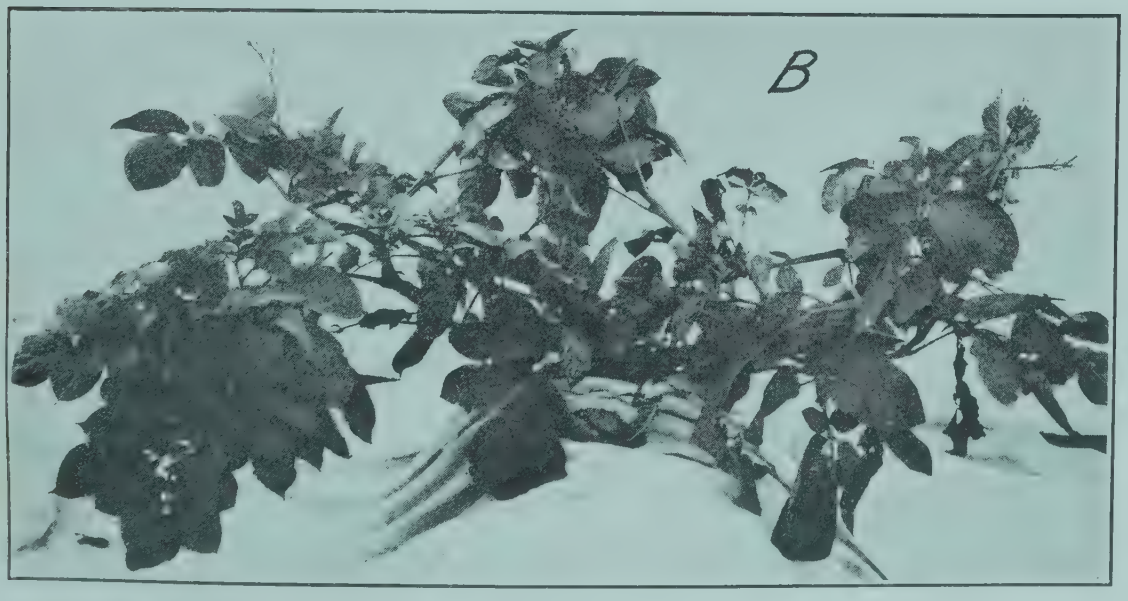
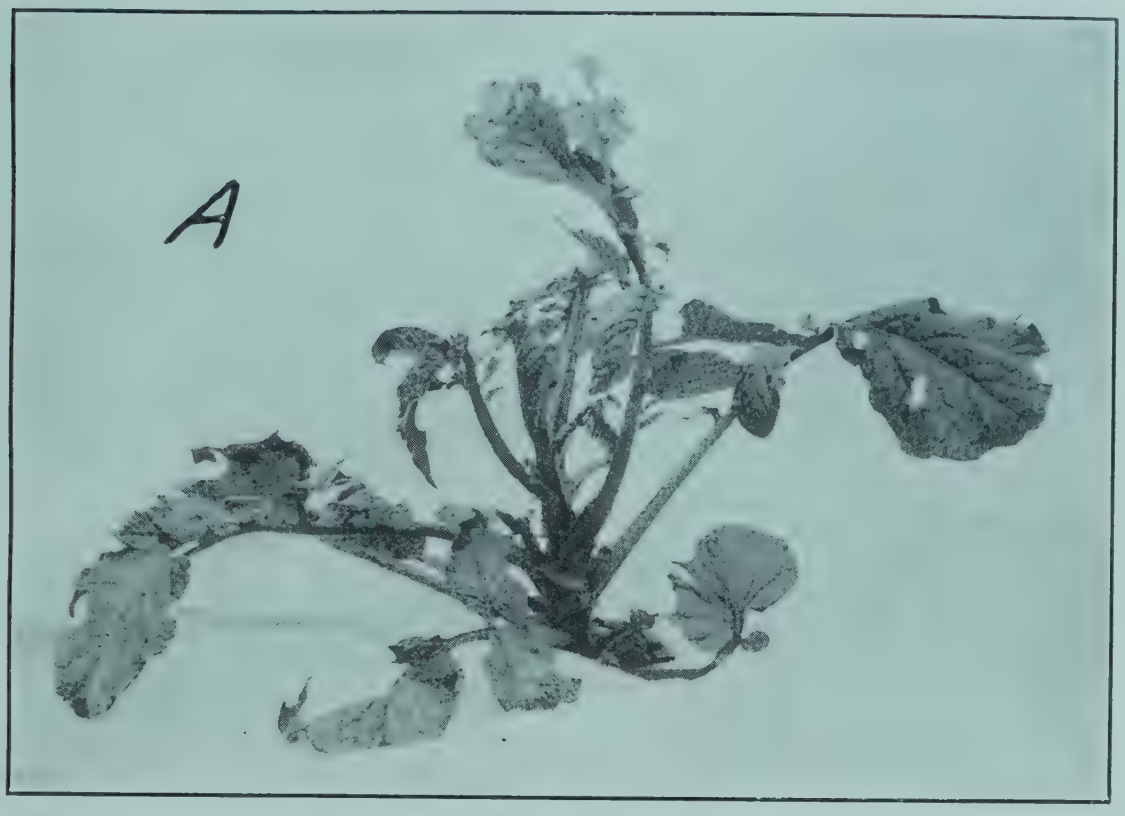

B

PLATE 10

Irish Cobbler greenhouse-grown plants from halves of the same tuber. The one on the right has leafroll due to inoculation with aphids from a diseased plant. Its greater height is due to the seed piece having earlier sprouts. The control on the left was fed upon by aphids from mustard plants but remained healthy.

PLATE 11

A.—Second-generation progeny of uninoculated control shown in Plate 10,
B.—Second-generation progeny of inoculated stock shown in Plate 10.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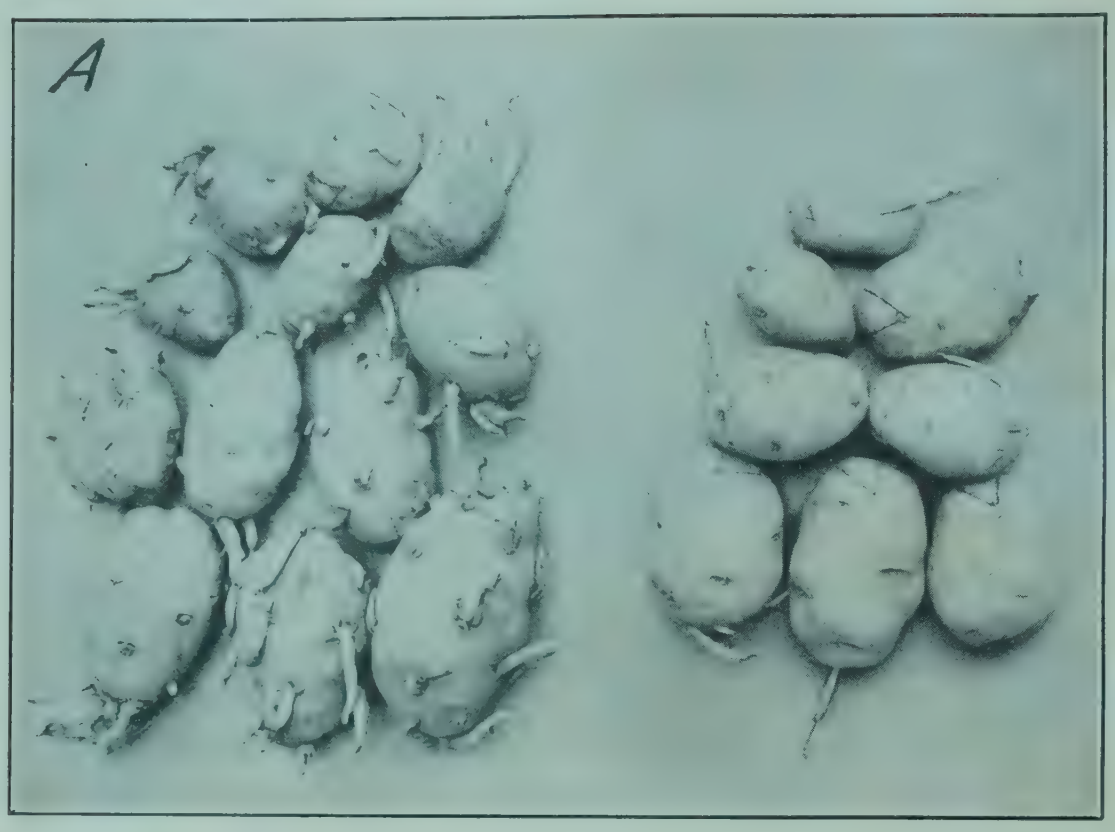
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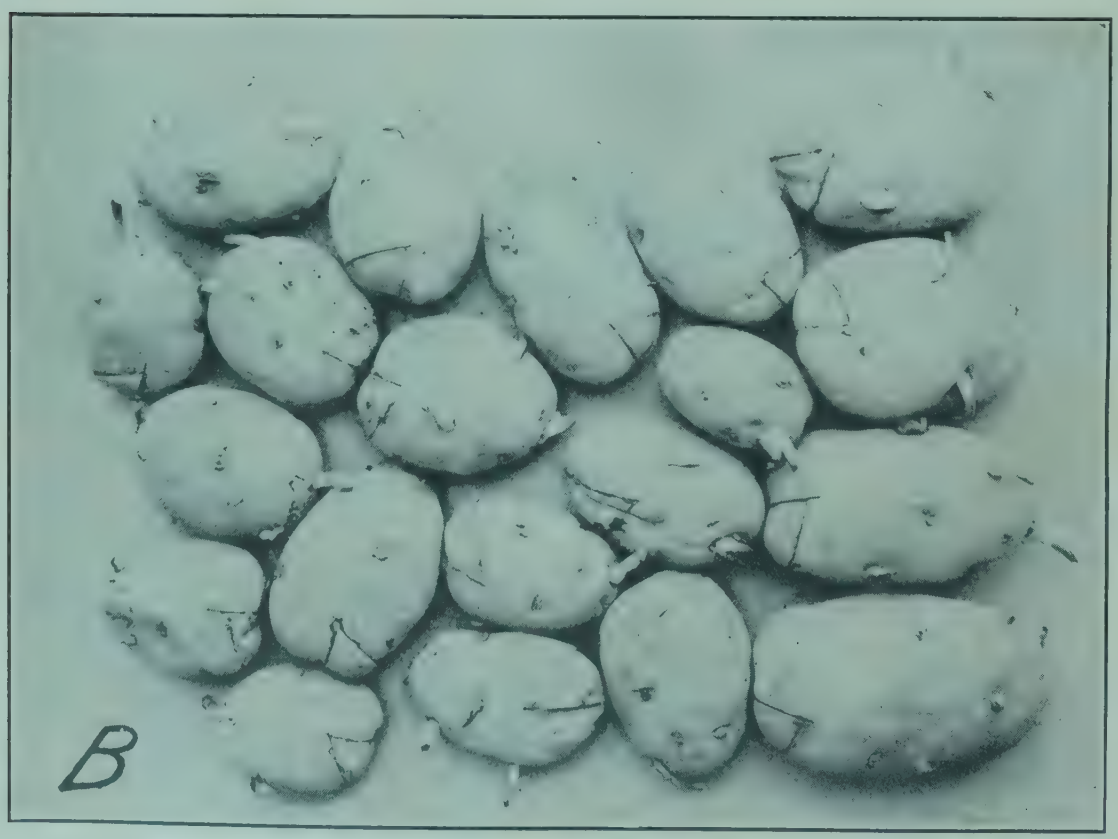
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PLATE 12

A.—New White Hebron tubers from 1919 hills next to leafroll hills in the same row. (Half of lot V148, sublot 3, of Table VIII.) Examined for net-necrosis and photographed in the last week of May, 1920, in northeastern Maine. The group on the right showed net-necrosis, spindling-sprout, and reduction in the number of sprouts and produced leafroll plants. Representative tubers from the two groups are shown in section in Pl. 3, B. From same stock and row as tubers of Pl. 12, B.

B.—New White Hebron tubers from hills leafroll throughout 1919. (Lot V148, sublot 2, of Table VIII.) Examined and photographed in the last week of May, 1920, in northeastern Maine. From same stock and row as tubers of A, and like them showed suppression of sprouts, but in contrast showed no net-necrosis or spindliness.

GROWTH AND SAP CONCENTRATION [1]

By HOWARD S. REED,[2] *Professor of Plant Physiology, University of California*

Chandler (*1*)[3] found that trees making rapid growth had lower sap concentration and that slower growth of the tree was accompanied by higher concentration. Trees which made rapid growth as a result of severe pruning in the preceding winter had a lower concentration of sap than trees which had been lightly pruned. During the summer season the concentration of the cell sap was lowest near the ground and highest in the upper part of the tree. Although Chandler's method of preparing the samples for freezing-point determinations has been adversely criticized, one must acknowledge the advance he made in our knowledge of sap concentrations in fruit trees.

Dixon and Atkins (*3*) observed that the sap of young leaves of various plants had, as a rule, a lower osmotic pressure than sap from mature leaves.

Lutman (*10*) has determined the freezing-point depressions of the sap of the potato plant with reference to its growth cycles. The variations of concentration seem to be related to the development and rate of growth of the plant.

The seed tubers when taken from storage had a relatively high osmotic pressure, but as water was absorbed the osmotic pressure dropped. As the age of the plant increases its osmotic pressure increases on account of the accumulation of inorganic salts in its cells; but when senility begins, the osmotic pressure drops, because of the loss of soluble materials. In the young plant the leaf sap is more concentrated than the stalk sap, but after the flower buds are put out and the tubers begin to grow, the stalk sap is more concentrated. With the advent of cool, rainy weather later in the season the leaves begin to grow again and their sap concentration is greater than that of the stalks. Shading the potato plant diminishes the osmotic pressure of the leaves and stalk.

Synopses of the earlier literature dealing with cryoscopic methods and with the relation of the physical environment to the concentration of the cell sap will be found in the summaries prepared by Dixon (*2*), Hibbard and Harrington (*7*), and Harris and Lawrence (*5*).

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[1] Paper No. 71, University of California, Graduate School of Tropical Agriculture and Citrus Experiment station, Riverside, Calif.

[2] It is a pleasure to acknowledge the aid which has been rendered by those associated with the progress of this work. The author's thanks are due to Messrs. L. C. Masters, R. H. Holland, and F. F. Halma.

[3] Reference is made by number (italic) to "Literature cited," p. 98.

METHODS OF EXPERIMENT

The methods of preparing and examining the material have been adapted to the needs of the work here reported. As the work of making several hundred determinations progressed, a routine procedure was followed which stabilized, if it did not remove, the sources of error. The chief object of this work has been to study comparative concentration of sap as related to the growth rate. The results obtained, though not free from error, are comparable with each other and give a satisfactory picture of an important physiological relation.

The plant material was collected in the field and packed at once in a tight container. For small samples, the container was a quart fruit jar with a screw top. For large samples, especially constructed steel cylinders, 6 inches in diameter by 14 inch tall, were used. The open end of the cylinder was provided with an accurately fitting metal cap and rubber gasket. The cap could be drawn down tightly on the rubber gasket by means of three bolts, and the cylinder was thus effectively sealed.

The samples were always collected between 9 and 10 o'clock in the forenoon, in order to avoid the possibility of diurnal fluctuations in concentration. As soon as filled, the containers were brought to the laboratory and packed in an ice-salt mixture for the preliminary freezing which killed the protoplasm and increased the permeability of the material. The supply of ice and salt was renewed as occasion required. The containers remained in this mixture for 18 to 24 hours, to insure complete freezing of the material. When the containers were removed they were washed to remove all adhering brine and wiped dry. The material was removed and ground in a small hand mill and then pressed in a strong screw press. Not more than 20 minutes were required to grind a sample and express the sap. The mill and press were heavily tinned, and there was no apparent corrosive action of the plant juices on any part of them after continued use.

The expressed sap was received in small bottles which were closed with rubber stoppers and placed on ice, in case the sap was not to be immediately used. Samples which had stood for more than two or three hours were discarded. In spite of these precautions, there was some oxidation of the plant saps and probable change in concentration; but the error, if any, due to such changes was shared to approximately the same extent by all samples and is not believed to vitiate the results.

The freezing point of the sap was determined in the usual freezing apparatus with the use of a Beckmann thermometer. At least two determinations were made upon each sample, and the average of closely agreeing duplicates was taken as the freezing point. The osmotic pressures were calculated by the method given by Harris and Gortner (*4*).

The rate of growth of the trees was obtained by measuring the length of certain selected shoots at intervals of seven days. Each shoot bore a

numbered tag and was marked near the base with a line of India ink. The distance from the basal ink mark to the tip of the shoot was easily determined and recorded for each shoot.

GROWTH INCREMENTS AND SAP CONCENTRATION OF YOUNG WALNUT TREES

Determinations on the concentration of the cell sap of young walnut trees (*Juglans* spp.) were made at frequent intervals and compared with the increments in mean height of similar trees growing in the same rows. The measurements given in Table I were made upon a population of *J. regia* trees, of which the majority were propagated upon *J. nigra* rootstocks and the remainder on a variety of *J. regia* known as "Hardshell." The trees from which the material for investigation was taken were in their second season's growth. They stood in the nursery row in a fertile soil and were artificially irrigated. The first samples for determinations were taken June 8 and the last on November 12.

The growth rate of a portion of this group of walnut trees has been discussed in a recent publication (*12*) and has been shown to follow the course of autocatalytic reactions.

TABLE I.—*Mean growth increments and concentration of cell sap of walnut trees*

Date.	Mean height.	Increment of growth during preceding week.	Osmotic pressure of cell sap.
	Cm.	*Cm.*	*Atmospheres.*
June 12	62.6	12.6	16.5
19	66.6	4.0	13.5
26	73.7	7.1	18.4
July 4	87.2	13.5	9.7
11	100 8	13.6	11.4
23	125.9	[b] 15.0	10.4
30	136.7	10.8	10.8
Aug. 6	147.6	10.9	.....
13	153.4	5.8	17.8
20	[a] 160.9	[a] 7.5	.....
27	168.4	[a] 7.5	12.5
Sept. 4	176.7	8.3	9.2
10	184.2	[b] 8.7	8.9
17	188.1	3.9	10.2
24	191.3	3.2	18.8
Oct. 1	194.6	3.3	11.7
8	196.8	2.2	20.9
15	199.0	2.2	13.2
22	199.7	0.7	17.1
29	201.2	1.5	13.5
Nov. 5	201.2	0.0	24.4

[a] Interpolated values. [b] Calculated to 7-day basis.

The samples collected for freezing-point determinations always consisted of the total growth of a shoot of that particular season (*ab initio*) and included both stems and leaves. Toward the end of the growing season the stems were large and woody; nevertheless they were ground in the handmill and pressed like the other material. Most of the sap so obtained came undoubtedly from the cortical layers of the stems.

By referring to Table I the reader will see the nature of the results obtained in this work. The table shows the mean height of the trees (from the basal ink mark), the height increment from the preceding measurement, and the concentration of the cell sap expressed in atmospheres of osmotic pressure. The mean height increased from 62 cm. on June 12 to 201.2 cm. on October 29. The increments (calculated in each case to a 7-day basis) were far from uniform, ranging as they do from 0.7 cm. to 15 cm. The atmospheres of osmotic pressure of expressed

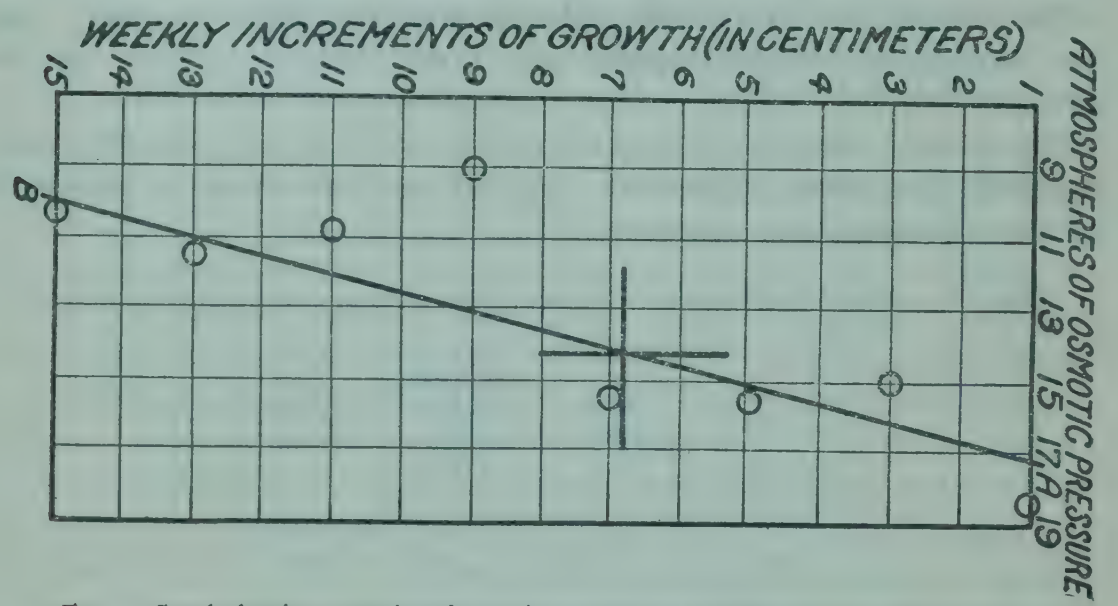

FIG. 1.—Graph showing regression of osmotic pressure on growth increments of walnut trees.

sap range from 8.9 to 24.4. Thus it will be seen that there are periodic variations in the concentration of the cell sap and in the growth rate from week to week during the season. The maximum growth rate was observed during certain weeks in July, and the maximum osmotic pressure of the cell sap was observed near the end of the growing season.

It is somewhat difficult to get a comprehensive idea of the relationship between growth and sap concentration from the array of figures given in Table I. It will be better to investigate the nature and amount of the mathematical correlation between the two sets of values. There are 19 observations in which both the increments and sap concentration were determined. While this number is rather scanty for determining correlation, the regression is remarkably linear (fig. 1), and it is believed that the correlation coefficient is trustworthy. The coefficient of correlation between the two variables is -0.557 ± 0.113. The negative

correlation shows that when growth is rapid the sap concentration is lower and when growth is slower the sap concentration is higher.

These conclusions may be verified by inspection of the figures in Table I. For example, an increment of 15 cm. in the week ending July 23 coincided with a sap concentration of 10.4 atmospheres, while smaller increments coincided usually with higher sap concentrations. The small growth increments in the latter part of the season are accompanied by the highest concentrations of plant sap. The concentration of solutes in the sap tends to increase with age of the leaf, as Dixon and Atkins (*3*) discovered in the study of Ilex.

GROWTH INCREMENTS AND SAP CONCENTRATION OF YOUNG APRICOT TREES (1918)

The question of sap concentration in shoots of young apricot trees in relation to growth was also investigated. The trees from which the material was obtained had been growing in the orchard two years when these studies were started. Soon after the season's growth began, 70 young shoots were selected and marked with labels. An India ink mark was made near the base of each shoot, from which length measurements were made at intervals of seven days. At the time of making the measurements here recorded a sample of shoots was collected from adjacent trees, and these samples were used for determining the sap concentration. The samples consisted of entire shoots of that particular season's growth. The stems and leaves were ground together, and the sap was expressed. The samples were given a preliminary freezing and were treated essentially as the walnut samples were treated.

The growth and sap concentration determinations made in 1918 upon apricot shoots are given in Table II. It will be noted that where the intervals were not exactly seven days the increments were calculated to a 7-day basis in order to make them comparable. It will be noted that the rate of growth was greatest at the outset of the observations and diminished with several fluctuations to the end of the growing season. Certain dynamical aspects of this growth rate have been discussed in a separate paper (*11*) though the studies there related were based upon another set of measurements.

The concentration of the cell sap (expressed in atmospheres of osmotic pressure), although subject to some fluctuation, increased as the season advanced. The lowest concentration was observed on May 21 and the highest on October 31. The average osmotic pressure, expressed in atmospheres by months, is: May, 11.84; June, 13.66; July, 14.34; August, 15.04; September, 15.18; October, 16.48. Table II shows that the concentration of the sap, which was 12.71 atmospheres on May 2, fell to 10.87 in the next three weeks, simultaneously with rapid elongation of the shoots. The concentration at once began to rise and went up, with some irregularities, to 18.90 atmospheres on October 31.

TABLE II.—*Mean growth increments of shoots and concentration of sap of apricot trees (1918)*

Date.		Mean length.	Increment of growth during preceding week.	Osmotic pressure of cell sap.
		Cm.	*Cm.*	*Atmospheres.*
May	2	45.0	19.0	12.71
	8	60.6	[b] 18.2	11.68
	15	74.1	13.5	11.99
	21	83.9	[b] 11.4	10.87
	29	92.4	[b] 7.4	11.95
June	7	103.9	[b] 9.0	12.30
	12	105.9	[b] 2.8	14.96
	19	108.5	2.6	13.52
	26	113.0	4.5	13.86
July	3	122.6	[b] 8.4	13.66
	10	134.2	11.6	14.24
	17	143.3	9.1	16.05
	24	148.0	4.7	15.28
	31	155.1	7.1	12.47
Aug.	7	162.2	7.1	14.87
	14	167.4	5.2	14.88
	21	170.0	2.6	15.17
	28	173.9	3.9	15.28
Sept.	5	[a] 184.2	[b] 9.1	15.54
	12	189.3	5.1	16.42
	19	192.3	3.0	13.46
	26	196.7	4.4	15.30
Oct.	3	199.3	2.6	15.64
	9	201.0	[b] 2.0	14.52
	16	203.2	2.2	14.99
	23	204.6	1.4	18.36
	31	206.8	[b] 1.9	18.90
Nov.	6	207.3	.5	17.66

[a] Interpolated value. [b] Calculated to 7-day basis.

The degree of association between the amount of growth in a week and the concentration of the cell sap at the end of that week is more succinctly expressed by the coefficient of correlation, which was found to be

$$r = -0.613 \pm 0.079.$$

This coefficient is of sufficient magnitude to express a strong negative correlation between growth and sap concentration. The regression of the two variables is approximately linear (fig. 2), though there is a marked tendency for the points to scatter. These determinations are based on 28 observations, however, and are regarded as somewhat more reliable than those for the walnut trees.

These observations, made at frequent intervals throughout a growing season, show that rapid growth is marked by a lower sap concentration and vice versa. In material of this kind, the concentration of the cell sap is probably due mostly to sugars and other organic compounds.

EFFECT OF PRUNING UPON SAP CONCENTRATION

The results obtained from determinations on the walnut and apricot trees are interesting and seem conclusive so far as they go, but they raise other questions which seem worthy of study.

In pursuance of these inquiries, determinations were made on the same block of apricot trees throughout the season of 1919. The growth and sap concentration were determined on two lots of trees, one of which is heavily pruned each winter while the other is not pruned at all. Needless to say, the growth of individual shoots on the heavily pruned tree, was much greater than that of shoots on the unpruned trees.

The mean length of shoots was determined at weekly intervals, as before. In the beginning, 50 shoots were selected on each lot of trees,

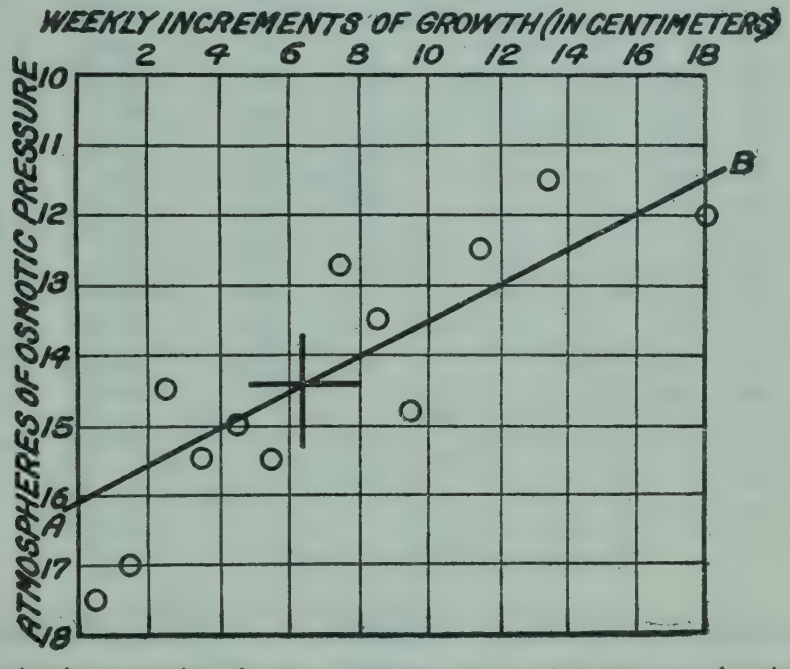

FIG. 2.—Graph showing regression of osmotic pressure on growth increments of apricot trees (1918).

but, as a result of breakage and other accidents, a number had to be eliminated. The computations are based on 33 shoots on the unpruned trees and on 28 shoots on the heavily pruned trees. The evaporating power of the air was determined by the evaporation of distilled water from a white, spherical atmometer bulb supported on a stand 5 feet from the ground in the center of the orchard. The average daily temperature was taken from the Riverside official reports, which were obtained from standard instruments located about 2 miles distant. The soil moisture was determined from composite samples taken to a depth of 3 feet throughout the area occupied by the trees in question. The sap concentration was determined by the depression of the freezing point.

A summary of the several factors is shown in Table III.

TABLE III.—*Growth and sap concentration of pruned and unpruned apricot trees (1919), with comparison of three environmental factors*

Date.	Time.	Evaporation.		Mean daily temperature.	Water added.[a]	Soil moisture.	Shoots on pruned trees.			Shoots on unpruned trees.		
		Per week.	Per day.				Mean length.	Increment of growth during preceding week.	Osmotic pressure.	Mean length.	Increment of growth during preceding week.	Osmotic pressure.
	Days.	*Cc.*	*Cc.*	*° F.*	*Acre-inches.*	*Per cent.*	*Cm.*	*Cm.*	*Atmospheres.*	*Cm.*	*Cm.*	*Atmospheres.*
Apr. 9	.....	.....	.....	.....	.....	.....	13	.....	.....	9	.....	.....
16	7	350	50	62	.....	.....	37	24	13.82	17	8	14.49
23	14	427	61	64	.....	.....	60	23	13.52	25	8	13.61
30	21	185	26	57	r 0.82	.....	73	13	11.72	29	4	13.41
May 7	28	192	27	61	i 3.40	.....	88	15	11.00	34	5	14.52
14	35	267	38	65	.....	.....	102	14	11.38	42	8	13.90
21	42	246	35	65	r .72	9.18	113	11	11.60	50	8	15.19
28	49	155	22	67	.....	9.43	121	8	12.75	57	7	13.97
June 4	56	508	72	73	.....	8.56	132	11	13.01	63	6	14.57
11	63	455	65	72	.....	8.39	142	10	13.82	68	5	16.73
17	69	366	61	69	.....	8.17	148	6	15.27	71	3	17.43
25	77	808	101	81	.....	7.53	156	8	14.76	77	6	15.94
July 2	84	447	64	73	.....	6.78	163	7	16.60	79	2	17.88
9	91	595	85	75	.....	5.60	174	11	.....	82	3	.....
16	98	501	72	78	.....	.....	177	3	15.87	83	1	19.77
23	105	.....	.....	75	i 2.70	.....	182	5	15.77	84	1	20.96
30	112	.....	.....	79	.....	7.22	186	4	18.51	85	1	21.81
Aug. 6	119	.....	.....	69	.....	6.02	190	4	14.09	86	1	17.14
13	126	496	71	76	.....	.....	194	4	.....	87	1	.....
20	133	483	69	79	i 2.81	5.48	197	3	16.38	88	1	19.50
27	140	334	48	77	.....	7.52	200	3	18.58	89	1	22.04
Sept. 3	147	263	38	74	.....	6.05	203	3	17.03	90	1	16.16
24	168	350	50	74	i 2.62	.....	208	2	19.66	94	1	21.94
Oct. 1	175	.....	.....	.....	r 1.37	.....	.....	.....	.....	.....	.....	.....
8	182	260	37	72	.....	5.92	210	1	22.73	94	0	19.40
13	.....	.....	.....	.....	i 3.80	.....	.....	.....	.....	.....	.....	.....
21	195	.....	.....	73	.....	6.66	.....	.....	.....	.....	.....	.....

[a] r = rain.
i = irrigation water.

The evaporation rate was at a maximum in June and July. During May the prevailing weather was cloudy, and the evaporation rate was thereby diminished. The mean monthly temperatures were as follows: April, 61° F.; May, 65°; June, 74°; July, 76°; August, 75°; and September, 74°.

The soil moisture diminished in quantity as the season advanced, in spite of the irrigations. The June irrigation was omitted on account of the ripening fruit. The figures show how the moisture content of the soil rose after each application of water.

The growth of the selected shoots on the pruned and on the unpruned trees was most rapid in the early part of the season. There were more or less distinct cycles of growth in each case, though they were more evident in the case of the heavily pruned trees. The increments in mean length are reduced to a 7-day basis. The mean growing season was approximately the same for each class of shoots, although the rate of growth of the unpruned shoots was very slow after the middle of July. Certain

dynamical features of the growth rate of these trees have been described in another paper (*13*) and will not be discussed here.

The concentration of the cell sap of the two classes of shoots fell off somewhat after the observations were started, then rose, although fluctuating, toward the end of the growing season. The concentration of the sap of the slower shoots on the unpruned trees was generally higher during the season than that of the rapidly growing shoots on the heavily pruned trees, although at the end of the season the sap of the two classes was substantially similar in concentration. Thus, both comparisons show that a higher concentration of cell sap is found in slower growing shoots.

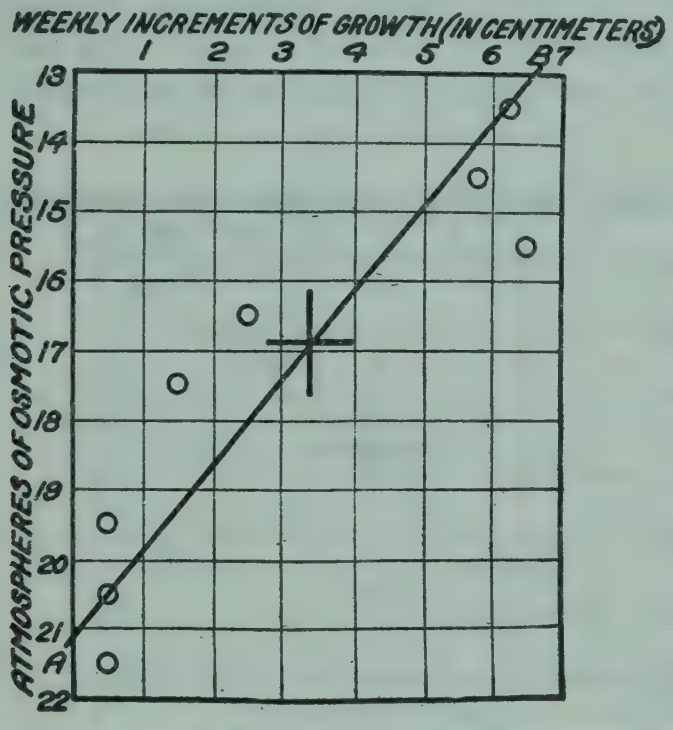

FIG. 3.—Graph showing regression of osmotic pressure on growth increments of unpruned apricot trees (1919).

In order to measure the association between growth and sap concentration I shall employ the correlation coefficient.

The correlation between concentration of cell sap and increment in length is

I.....$r = -0.628 \pm 0.088$ for heavily pruned trees.
II.....$r = -0.823 \pm 0.049$ for unpruned trees.

Reference to figures 3 and 4 shows that regression in the pruned trees is fairly linear, but in the unpruned trees this linearity is doubtful. In the latter case, the significance of the correlation coefficient may be more apparent than real.

It will be readily recognized that factors which contribute to the higher sap concentration of the unpruned trees are associated with the nature of the growth they made. Their slower growth and consequent diminished draft on the plastic materials of the tree should allow a greater

accumulation of soluble materials in their tissues. The smaller water content of the slow-growing wood also contributes to a higher concentration in the sap.

The increased concentration of soluble materials in the sap of both classes of trees as the season advanced is to be referred in part to the foregoing factors and in part to the increase in soluble organic products of photosynthetic activity. With the increase in leaf area and the advance of the season there should be an increase in the content of soluble carbohydrates in the cell sap.

The water content of the soil has an important influence upon sap concentration of the plant (compare Hibbard and Harrington, 7). There are numerous instances to be seen in the table where the sap concentration fell after the application of irrigation water; but it will be noticed that the drop in sap concentration was not evident until, in some cases,

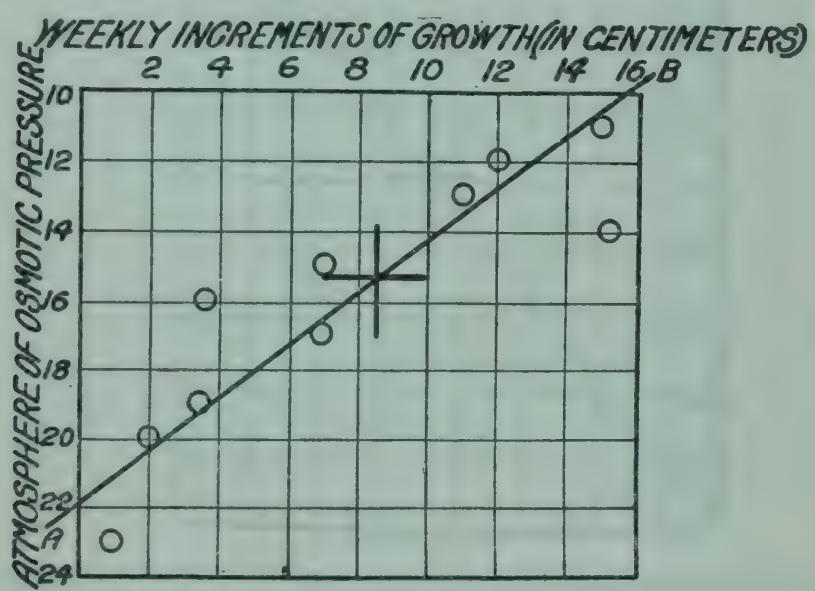

FIG. 4.—Graph showing regression of osmotic pressure on growth increments of pruned apricot trees (1919).

one or two weeks after the application of water. As the season advanced the amount of water in the soil gradually diminished, in spite of the irrigation, and there is little doubt that the gradually diminishing water supply is to some extent causally related to the gradually increasing concentration of the plant sap through the season. It is difficult to express the relationship by a correlation coefficient because the regression between the two pairs of values is not linear. After a certain moisture content (say that of saturation) is reached, a further addition of water would have no effect on the sap concentration of the tree. It may not, however, be entirely improper to attempt to express the relationship as a coefficient of partial correlation. This figure will express the relationship between growth increments and sap concentration, assuming the soil moisture to have been constant. In the case of the heavily pruned trees,

$$r_{ip \cdot w} = -0.525.$$

Taking account of the moisture factor thus reduces the coefficient from −0.628 to −0.525.

SAP CONCENTRATION IN APICAL AND BASAL REGIONS OF APRICOT SHOOTS

Determinations of sap concentration were made at weekly intervals between June 12 and December 4 in the leaves and stems of a 10-cm. zone at both base and apex of apricot shoots. The concentration, as determined by the lowering of the freezing point, is shown graphically in figure 5.

It will be seen that the sap in the apical portion of the stem was more concentrated than that in the basal region. Increases or decreases in the

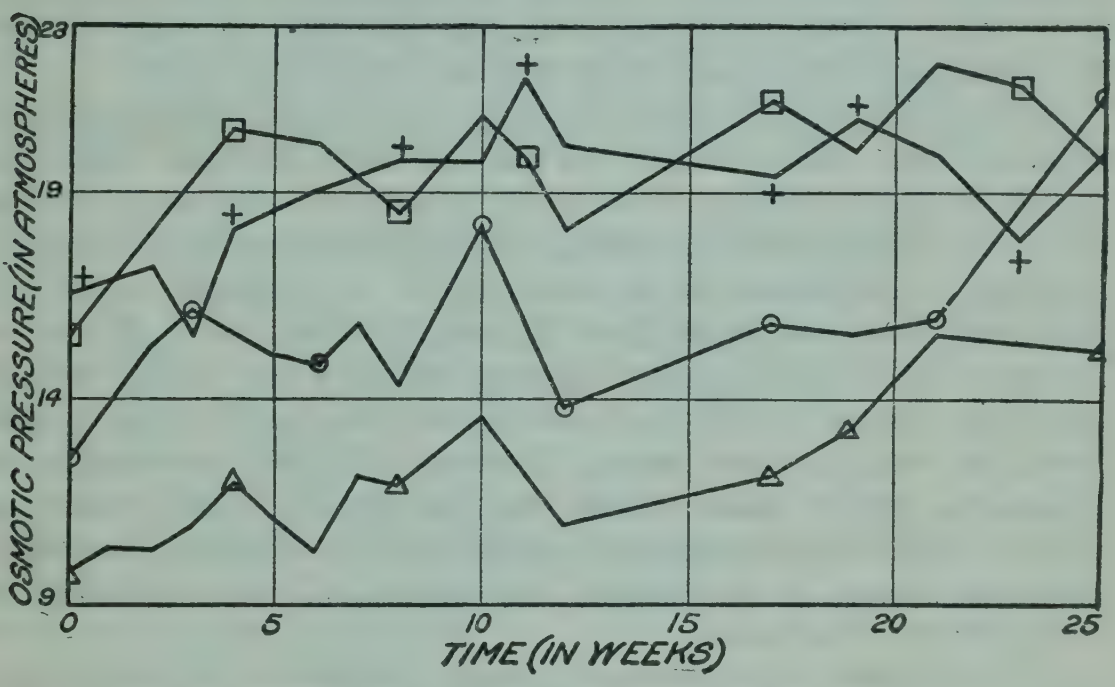

FIG. 5.—Graph showing seasonal variations in sap concentration in apricot shoots.
○=sap concentration of apical 10 cm. of stems.
△=sap concentration of basal 10 cm. of stems.
□=sap concentration of leaves from 10-cm. zone at apex.
+=sap concentration of leaves from 10-cm. zone at base.

one are approximately parallel to increases or decreases in the other. The greater divergence in the last observations is probably due to the dry condition of the samples and to the possibility of error in one or both determinations. In general, the differences between results were sufficiently great to indicate differences well outside the realm of experimental error.

The sap concentrations of the leaves from the different regions show no wide nor consistent differences, though there is a tendency for them to vary in opposite directions—that is, when one rises the other falls, and vice versa. In general, the sap concentration of the leaves was well above that of the stems at corresponding periods, a relation which was pointed out by Heald (*6*).

TABLE IV.—*Mean and root-mean-square deviations of sap concentration in apical and basal regions of apricot shoots*

Part of shoot.	Mean sap concentration.	Root-mean-square deviation.
	Atmospheres.	*Atmospheres.*
Stem, apical	15.83	2.06
Stem, basal	12.59	2.77
Leaves, apical	19.59	1.74
Leaves, basal	18.93	1.68

The mean sap concentrations, shown in Table IV, bear out the facts represented by the graphs. The sap concentration at the apical end of the shoot was higher in both stem and leaf, although there is very little difference in the leaves at the opposite ends of the shoot.

The root-mean-square deviations show that the sap concentration of the stem had a greater tendency to fluctuate about its mean value than that of the leaf. This may be taken to indicate that the sap concentration of the leaves tends to maintain an equilibrium which is not easily disturbed by fluctuations in the environment. The variation in the sap concentrations of the leaves from the middle of July on to the end of the season is within the range of experimental error. Other investigators have found that the young leaves on a plant had a lower sap concentration than the older leaves. A similar condition may exist in the apricot tree, but it would require that one should carefully select the leaves in order to demonstrate it. At the time the foregoing determinations were begun on the apricots, the shoots had made two-thirds of their growth for that season.

It thus appears that there is a gradual increase in concentration of sap from the base to the apex of a growing shoot.

There is, in some quarters, a belief that the apical leaves on young shoots are meagerly supplied with solutes and that the practice of removing the terminal half of the young shoots is justifiable at any time after mid-summer. The determinations made upon these apricot shoots seem to speak against such a belief. From the middle of the summer to the first of December there was little real difference in the sap concentration of the leaves at opposite ends of the new shoots.

The existence of a gradient in the sap concentration of apricot shoots was shown by determinations made on June 10, 1920, using a sample of young shoots having an approximate length of 120 cm. The leaves were removed and the stems were cut into four lengths of 30 cm. each. The sets were designated as A, B, C, and D. A designated the apical set of cuttings and D the basal set.

Sap-concentration determinations were made upon each of these samples separately. The concentrations expressed as atmospheres of osmotic pressure were as follows:

A	13.19
B	11.81
C	10.97
D	10.32

CONCENTRATION OF SAP IN ORANGE SHOOTS AND LEAVES

The leaves of the orange tree persist for more than one year. New shoots commonly arise from axillary buds and may attain considerable length before the subtending leaf falls. Determinations were made of the sap concentration in new shoots of the Washington Navel orange and of the old leaves subtending them. The leaves had grown to maturity in the summer preceding the appearance of the shoots and might be supposed to have some physiological relation to their axillary shoots.

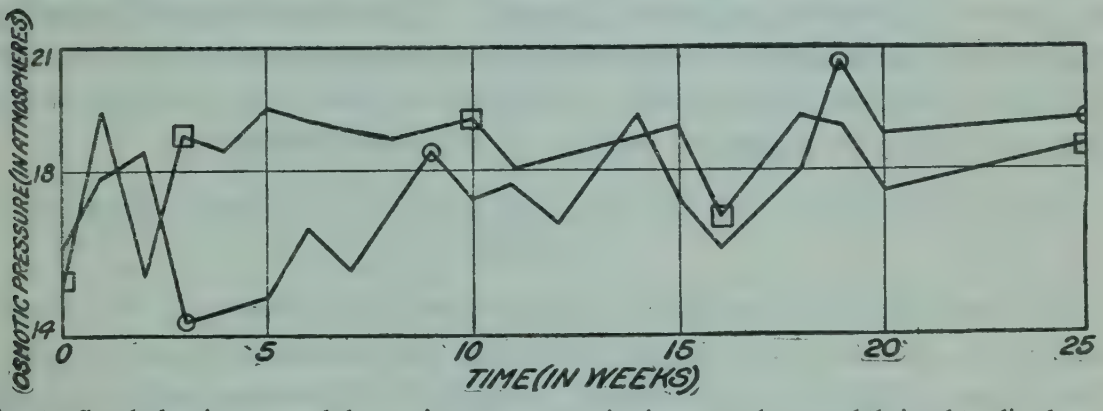

FIG. 6.—Graph showing seasonal changes in sap concentration in orange shoots and their subtending leaves.
○=sap concentration of young shoots.
□=sap concentration of the subtending leaves.

There was, however, no general relationship indicated by the sap concentration of the two members. Figure 6 represents graphically the changes in sap concentration of leaves and their axillary shoots from April 15 to October 9. During the first few weeks the sap concentration of the stem appeared to fluctuate and reached the low point for the season about May 1. From May until October there was a general tendency for the sap concentration to rise, though not to so marked a degree as was seen in the apricot. Throughout most of the summer the concentration of the leaf sap was greater than that of the shoot sap. The mean of the determinations of the leaf sap was 18.4 atmospheres and of the shoot sap 17.3 atmospheres.

The root-mean-square deviation of a series of values from their mean, as stated above, is an excellent measure of their dispersion. In this case the root-mean-square deviation of the leaf-sap concentration is 1.21 atmospheres and that of the shoot sap is 1.64 atmospheres. This shows that the concentration of sap in the leaves was subject to slightly less

fluctuation during the period of the observations than that of the shoots. In all cases these leaves were not more than 1 year old and were performing the functions common to orange leaves of their age. It seems difficult to escape the conclusion that in some way the concentration of solutes in the leaves tends to reach and maintain an equilibrium, and that this equilibration occurs regardless of the variations in concentration which simultaneously take place in the adjacent shoots.

CONCENTRATION OF THE SAP IN VARIOUS LAYERS OF THE APRICOT SHOOTS

The following osmotic pressures were found in a sample collected on June 27: mature leaves, 17.12 atmospheres; cortical layers of the stem, 13.82 atmospheres; woody, subcortical layers, 10.21 atmospheres. These figures indicate that the sap of the wood is less concentrated than that of the leaves and are in entire agreement with those previously obtained. They show quite remarkable differences in the sap concentration of the different layers of the stem. The cortical layers (including the phloem) undoubtedly owe their higher concentration to their content of plastic organic substances. Although subject to variation, these relations might be expected to hold throughout the season.

SAP CONCENTRATION OF THE APRICOT TREE AFTER GROWTH CEASES

It is well known that during the progress of "ripening" the woody tissues are the seat of changes involving the translocation and deposition of many kinds of compounds. A series of determinations was made after the shoots on these trees ceased to show further elongation to see whether there were appreciable changes in sap concentration in the early part of the dormant period.

TABLE V.—*Sap concentration of apricot shoots during fall and early winter*

Date.	Osmotic pressure.	
	Unpruned trees.	Heavily pruned trees.
	Atmospheres.	*Atmospheres.*
Oct. 23	18.60	15.81
Nov. 6	19.10	17.90
20	19.00	17.20
Dec. 4	16.33	17.22
18	19.10	22.30
Jan. 29	15.85	18.75

These figures show that there are appreciable changes in the concentration of the sap even though no active growth is taking place. The changes may, however, be referred in part to changes in the amount of

soil moisture present. During November and December there were light rains which did not penetrate deeply into the soil and did not affect the concentration of the cell sap of the trees. On January 4, 0.585 inches of rain fell, and on the three succeeding days 5.2 acre-inches of irrigation water were applied. The effect of this increased supply of soil moisture is seen in the lower concentration of sap in the samples taken on January 29. The effect of the increased amount of water intake was to dilute the sap of the tree, even though active growth had ceased.

SAP CONCENTRATION AND FRUITFULNESS

The observations made upon these fruit trees may throw some additional light upon the relationships between vegetative and fruiting activity of the tree. It appears that lower sap concentration is associated with abundant water intake and rapid growth. Severe pruning, which also stimulated active vegetative growth, caused *pari passu*, a lowering of the concentration of sap in the tree.

Horticulturists have long recognized that those conditions which are associated with lower sap concentration are opposed to fruit bearing in a tree. With the data now in hand it seems possible to point out some additional relationships of interest. The most rapid growth occurs in the early part of the season. The apricot shoots in 1919 made about half of their total growth in the first one-fifth of their growing period. By the time that three-fourths of the length growth had been made, the sap concentration had reached a fairly high value, which was maintained during the remainder of the season. Now it is well known that this time of higher sap concentration is the time during which the fruit buds for the following year are developing on the tree. The various lines of evidence lead to the conclusion that higher sap concentration is associated with slower growth and fruit-bud formation. At the present time, however, it seems impossible to say which one of the conditions is cause and which is effect. Differences in the sap concentration of the stem at the apical and the basal ends of the shoots are so well marked as to be unmistakable, yet it is somewhat difficult to orient one's views to fit the facts. The sap concentration of entire shoots, including stems and leaves, was lower during periods of rapid growth and higher during periods of slower growth. Yet, when we note that the sap concentration of the rapidly growing apical region of the shoot was uniformly higher than that of the slowly growing basal region, the condition seems paradoxical. The condition seems the more paradoxical when one remembers that the apical portion of the stem has a higher water content and that the content of solutes must, therefore, be correspondingly higher in order to show a higher concentration in the expressed sap.

We should remind ourselves, however, that growth is not wholly regulated by concentration *per se*. The composition of the sap, no less than

its total concentration, affects the activity of the cell. Attention has recently been called to the effect on plant growth of inhibiting substances resembling chalones (*9*, *14*). The absence of these growth-inhibiting substances in the apical portion of the shoot accounts in large measure for the more rapid growth of that region. There are also numerous lines of evidence which indicate that the sap of the apical region of the stem promotes vegetative growth because of its greater content of nitrogenous substances, while the slowly growing basal portion of the shoot is favorable to fruit-bud formation because of its greater content of carbohydrates (*8*). It goes without saying that we must recognize qualitative as well as quantitative differences in the plant sap in analyzing its

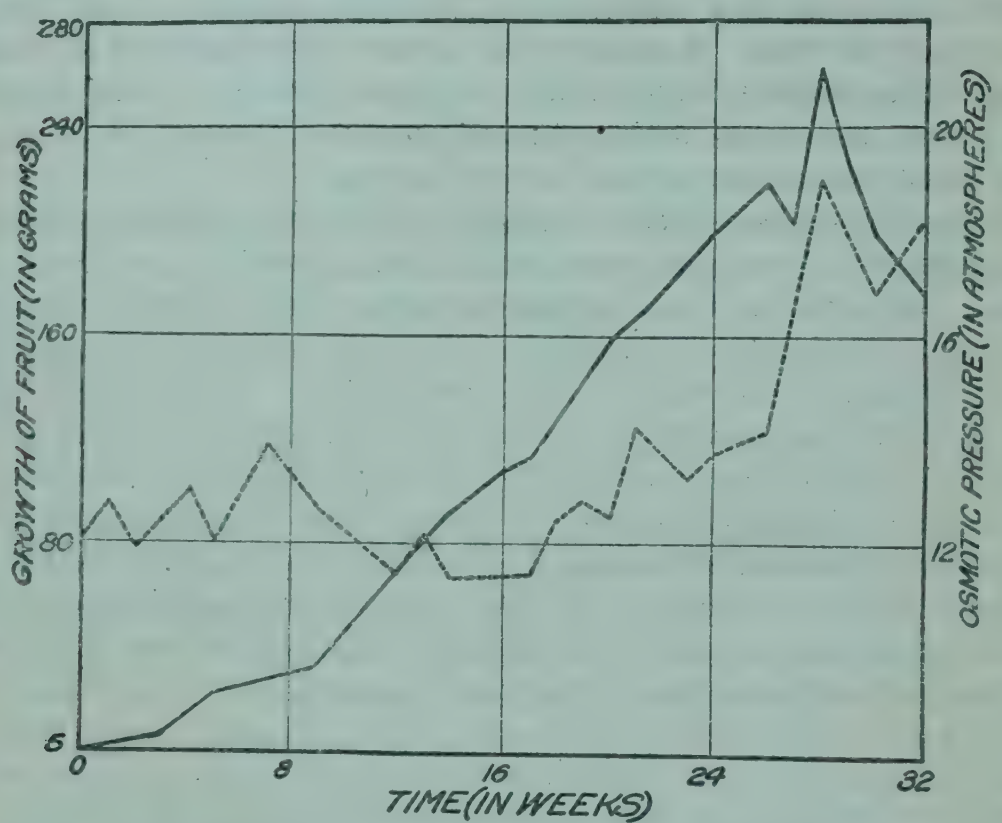

FIG. 7.—Graph showing growth and sap concentration of Golden Nugget navel oranges. Average weight of fruits is shown by solid lines, sap concentration of fruits by broken line.

effects on growth. The evidence, however, indicates that an increasing sap concentration in the shoot as a whole retards vegetative growth, while a decrease in the sap concentration of the whole shoot favors growth.

SAP CONCENTRATION AND THE GROWTH OF ORANGE FRUITS

The orange fruit was selected for study because it has a relatively long period of growth and develops on a tree which continuously bears green foliage. The variety employed is a strain of the navel orange known as "Golden Nugget." Determinations began on June 5, 1918, and were continued at weekly intervals until January 16, 1919. The mean weight of fruits on the former date was 0.39 gm. The growth rate was rather slow from the beginning of the observations until the end of the ninth

week; then a period of more rapid growth set in, which lasted until the twenty-sixth week (Dec. 1). Toward the close of the period there was considerable fluctuation in the weight of fruits, largely because of errors in sampling, but the final mean weight was somewhat over 200 gm. The courses of the growth process and of the sap concentration of the fruit are shown in figure 7. During the first nine weeks, while the fruits were growing slowly, their sap concentration varied between 12 and 14 atmospheres of osmotic pressure. When more rapid growth began, the sap concentration dropped to a pressure between 11 and 12 atmospheres, but subsequently gradually rose. As the fruit approached maturity there was a great increase in its sugar content and a consequent increase in the concentration of the sap.

During the first half of the growth period it is quite evident that rapid growth is accompanied by a lower sap concentration. In the latter half of the growth period it is more difficult to ascertain the relationships between sap concentration and growth because of the fact that while the fruit is still growing it begins to accumulate increasing amounts of soluble carbohydrate.

SUMMARY

(1) Observations on the growth and sap concentration of young trees showed that the two variables have a tendency to vary in opposite directions—that is to say, rapid growth is associated with a generally lower concentration of sap in that shoot, while slower growth is accompanied by higher concentrations of sap.

(2) There was a gradual increase in sap concentration as the season advanced. In apricot trees the concentration continued to increase for some time after active growth had ceased. The accumulations of solutes in the plant sap is unquestionably related to the synthetic metabolism of the tree as the season advanced, though there is some evidence that diminished water absorption was partially responsible for the increased sap concentration.

(3) Of several environmental factors measured, soil moisture was the only one having an obvious effect upon sap concentration. The addition of water to the soil usually diminished the concentration of the plant sap.

(4) The sap concentration of shoots on trees heavily pruned was lower than that of shoots on trees not pruned, because of the more rapid growth of the former.

(5) A concentration gradient appears to exist in the shoot. The concentration of the sap in the apical portion of a stem was greater than that in the basal region. The sap concentration in the stems showed a greater tendency to fluctuate than that in the leaves.

(6) Lower concentrations of plant sap in the shoot as a whole appear to be associated with abundant water intake and rapid vegetative growth.

Higher concentrations are associated with slow growth and fruit-bud formation.

(7) The results seem to indicate that the practice of summer pruning fruit trees is not only unnecessary but may be detrimental.

(8) Observations upon the growth and sap concentration of shoots and fruits of the orange tree confirm those made on the apricot tree.

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COTTON ROOTROT IN THE SAN ANTONIO ROTATIONS

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INTRODUCTION

The disease known as cotton rootrot has been prevalent on the San Antonio Experiment Farm since cotton experiments were begun there in 1906. It was not, however, until 1912 that definite observations on the occurrence of the disease were made a matter of record. In that year counts were made of the number of cotton plants affected by rootrot in certain plots in a series of crop-rotation experiments, and the proportion of these diseased plants to the total number of plants in the plots was computed. Similar observations have been made each year since 1912, so that with the season just passed there are available eight years' records of the disease in these rotation fields.

In 1916 the disease was so serious that more detailed observations concerning it seemed justified, and at the close of that season each of the cotton plots in the rotation fields was carefully measured and a diagram was made, showing the spots in which the plants were dead from the effects of the disease. Each season since 1916 similar diagrams have been made of each cotton plot.

It has been thought that the best remedy for cotton rootrot was to be sought through a suitable rotation of crops, supplemented by deep plowing and thorough tillage. The rotation experiments here considered include a number of different crop sequences as well as some very different methods of tillage. While these rotation and tillage experiments were not planned with particular reference to rootrot control, they afford an opportunity to observe the reactions of the disease to different treatments during a series of years.

During the eight years of record, 1912 to 1919, the disease has become increasingly serious in these rotation plots, as is shown in Table I, which gives for each year the average percentage of plants killed by rootrot in all the cotton plots in the rotation experiments.[1] There were 25

[1] The actual increase in severity of the disease has been somewhat less than the data here given would indicate, for the reason that prior to 1916 the rootrot counts were made before the end of the growing season. The actual dates on which the counts were made are as follows: 1912, Aug. 13; 1913, Sept. 4; 1914, early September (exact date not recorded); 1915, Sept. 28; 1916, Oct. 23 to 25; 1917, Oct. 25; 1918, Oct. 23 to 29; 1919, Oct. 27 to 31.

cotton plots in 1912 and 30 plots devoted to this crop each year since 1913. Of this number, 4 have been in cotton continuously, 20 have been in cotton each alternate year, 4 have been in cotton every third year, and 2 have been in cotton every fourth year.

The table also gives the average yield of all cotton plots in the rotations in pounds of seed cotton per acre, the total annual precipitation as recorded at the experiment farm, and the precipitation occurring during the crop season each year from April 1 to October 31.

TABLE I.—*Average percentage of cotton plants killed by rootrot in the rotation experiments at San Antonio, Tex., the average yield of these plots in pounds of seed cotton per acre, the total annual precipitation, and the seasonal precipitation, April to October, for the years 1912 to 1919*

	1912	1913	1914	1915	1916	1917	1918	1919
Average percentage of plants killed by rootrot	0.4	1.3	2.0	7.1	21.4	9.7	12.7	26.0
Average yield in pounds of seed cotton per acre	621	560	784	567	430	536	259	42
Total annual precipitation	26.29	36.71	31.37	26.64	27.26	13.22	27.06	47.64
Total seasonal precipitation	11.67	22.77	22.98	21.22	21.47	11.18	19.05	38.12

DESCRIPTION OF THE ROTATIONS

The plots on which these rotation experiments are conducted are ¼ acre in size, being 264 feet long by 41.25 feet wide. The longer dimension of the plots lies on an east and west line, and the alleys between the plots are 4.75 feet wide. When planted to cotton each plot contains 10 rows, 4.1 feet apart. The rotation plots are arranged in two series which extend north and south. Of these series the one on the west is called series A and the one on the east series B. A field road some 20 feet wide runs along the west side of series A, another separates the series from each other, and still another north and south road bounds series B on the east. Each series is subdivided by two crossroads, 16 feet wide, so placed that each subdivision of a series includes 18 quarter-acre plots. The subdivision or field at the north end of each group is numbered 4, the center one is numbered 5, and the one at the south end is numbered 6. Thus, field A–4 lies at the northwest corner of this group of six fields, and field B–6 lies in the southeast corner. The plots in each field are numbered from 1 to 18. The regularity of the system just described is broken in field A–4 by placing 4 plots whose long dimensions lie on a north and south line in the space that should be occupied by the 3 plots, 7, 8, and 9. These 4 plots—numbered 7, 8, 9, and 10, counting from east to west—are each surrounded by a ridge or border to prevent the run-off of water during torrential rains. Consequently there are in field A–4 actually 19 plots instead of 18, as in the other fields.

This detailed description of the location of these field plots is given so that the reader may the better interpret the subsequent notes on the field distribution of the rootrot infection.

It is not necessary for the present purposes to describe all the rotations in the San Antonio series, so that only those including cotton are given in the following list, and only the principal features of the tillage methods preceding the cotton crop are indicated. These cotton rotations may be grouped into four categories, as follows:

1. Cotton continuously:

A–4–19. Cotton following field peas plowed under in March.
A–6–3. Cotton on land plowed in November.
B–5–3. Cotton on land plowed in November.
B–5–4. Cotton on land plowed in November and manured each year at the rate of 16 tons per acre.

2. Cotton on the same land alternate years:

A–4a. Cotton on land plowed in December and fallowed for 16 months.
A–4d. Cotton following corn on land plowed in July and fallowed 8 months.
A–5c1. Cotton following oats on land plowed in June and fallowed 9 months.
A–5c2. Cotton following grain sorghum on land plowed in July and fallowed 8 months.
A–5d1. Cotton following cowpeas plowed under in November after an oat crop maturing in June.
A–5d2. Cotton following grain sorghum on land manured and plowed in July and fallowed 8 months.
A–5e1. Cotton following cowpeas plowed under and the land subsoiled after an oat crop maturing in June.
A–5e2. Cotton following grain sorghum on land manured, plowed, and subsoiled in July, and fallowed 8 months.
B–5a. Cotton on land plowed in November following forage sorghum.
B–5b. Cotton on land plowed in November following forage sorghum.
B–5e. Cotton on land plowed in July following grain sorghum.
B–6a. Cotton on land plowed in July following corn.
B–6b. Cotton on land plowed and subsoiled in July following corn.
B–6c. Cotton on land manured and plowed in July following corn.
B–6d. Cotton on land following corn, manured, planted to field peas, and plowed in March.
B–6e. Cotton following corn, manured, planted to field peas, plowed under in March, and subsoiled after the cotton crop.
B–6f. Cotton following corn on land disked in July after the corn crop but not plowed until after the cotton crop.
B–6g. Cotton on land plowed in February after corn.
B–6h. Cotton on land plowed and subsoiled in February after corn.
B–6i. Cotton following corn with rye disked in after the corn and plowed under in February.

3. Cotton on the same land every third year:

A–5a. Cotton on land plowed in June, fallowed 9 months following oats for hay, which had followed grain sorghum.
A–5b. Same as A–5a, except that the land was manured following the cotton crop.
B–4a. Cotton on land plowed in November following Sudan grass, which in turn followed grain sorghum.
B–4b. Cotton following field peas plowed under in March after oats for hay which followed grain sorghum.

4. Cotton on the same land every fourth year:

A–4f. A rotation of oats for grain, land plowed in June; grain sorghum followed by field peas plowed under in March; forage sorghum, land plowed in November; cotton planted the following April.
A–4g. A rotation that differs from the above only in that the field peas are cut for hay instead of being plowed under.

The plowing in these rotations is ordinarily done to the depth of about 8 inches; where subsoiling is indicated, this is done in each furrow after the plow to the depth of 12 to 14 inches. After plowing, all plots are kept in good tilth and free from weeds by harrowing and disking until the subsequent crop is planted. Where manure is indicated, it is applied at the rate of 16 tons per acre and plowed under.

It will be observed that cotton is the only crop used in these rotations that is subject to rootrot injury and that the diversity of tillage methods fairly well covers the range of farm practice in this region.

In the first of the categories listed above, cotton recurs on the same plot each year. In the second case, cotton recurs on the same plot each alternate year, so that to give an annual expression as to the rootrot injury each rotation may be regarded as occupying a field of ½ acre in size, one-half of which is in cotton each year. Likewise, for the third category, each of the four rotations may be thought of as occupying a plot of ¾ acre in size, of which one-third is in cotton each year, permitting an annual expression of the rootrot injury for each rotation. In the fourth case the rotation would be regarded as covering 1 acre of ground, of which one-quarter is in cotton each year.

This method of comparison is faulty, of course, in that it assumes a degree of uniformity as between adjacent plots with respect to rootrot infection that probably does not exist. But, on the other hand, in view of the fact that the different rotations were located in the fields without previous knowledge as to the location of rootrot areas there was no chance for bias in their distribution. It would be fair to assume that if any system of rotation or any method of tillage were really effective in controlling the disease this effect would be shown by a lower annual percentage of plants dying from the disease within the area subjected to that system or method.

COMPARISON OF THE ROTATIONS

In order to compare the effect of the disease as observed in the different rotation plots, it is necessary to present in some detail the annual records as to the percentage of plants killed by the disease.

In the first of the categories listed above there are four plots on which cotton has been grown continuously during the period of record. The extent of rootrot injury in each of these plots is shown in Table II.

TABLE II.—*Percentage of plants killed by rootrot in the continuously cropped cotton plots at the San Antonio Experiment Farm, 1912 to 1919*

Plot No.	1912	1913	1914	1915	1916	1917	1918	1919	Plot mean.
A–4–19	(a)	2.7	4.2	11.8	42.0	10.6	25.6	32.3	18.4
A–6–3	0.7	.8	.5	.7	7.4	15.1	9.2	19.5	6.7
B–5–3	.9	3.8	17.6	49.7	96.2	43.7	30.3	95.5	42.2
B–5–4	.2	.8	2.5	10.5	60.5	36.8	42.0	74.3	16.0
Annual mean	.6	2.0	6.2	18.2	51.5	26.5	26.8	55.4	.....

[a] Not planted to cotton in 1912.

This tabular statement shows that these continuously cropped cotton plots have all suffered severely from rootrot. As has been the case with all the plots in the rotations, the disease has become increasingly serious during the later years. The two plots that have had identical treatment, A–6–3 and B–5–3, show wide differences in extent of infection, but this may be due in part to situation, as they are in different parts of the field. The plots B–5–3 and B–5–4 lie side by side. Their tillage treatment has differed only in that B–5–4 has received annual applications of manure. It is not clear that this difference in treatment is responsible for the difference in results, but it may be a contributing factor. It is clear, however, that in A–4–19 the annual use of a green manure crop of field peas plowed under has not been strikingly effective in controlling the disease.

THE TWO-YEAR ROTATIONS

The second category of rotations includes those in which cotton recurs on the same land each alternate year. The extent of rootrot injury in these 20 two-year rotations is shown in Table III.

The outstanding feature of Table III is the wide variation in the extent of rootrot injury in these different rotations, a range in means from 0.8 per cent to 31.9 per cent. If there were reason for believing that these differences in rootrot injury were associated with differences in tillage methods or with different crop sequences, it would appear that the remedy for the disease was to be sought through the adoption of the tillage and rotation methods which have shown the least injury through the period of years. But, unfortunately, a critical study of the facts does not warrant such a conclusion. It is only by detailed analysis of the facts involved that the real significance of the results can be understood. For such a detailed study it may be expedient to separate these 20 rotations into groups with respect to some significant feature of the tillage or rotation practice, as follows.

1. LONG PERIOD OF FALLOW.—Rotation A–4a, cotton grown on land in alternate years, the land being plowed immediately after the crop is picked and kept in clean fallow for about 16 months until the next cotton crop is planted. The history of this rotation shows that very little rootrot developed prior to 1915 but that the injury has been severe during 1918 and 1919. This rotation may be compared directly with A–6–3 and B–5–3, which differ from it only in the length of fallow period, this being 16 months in the first case and 4 months in the other two cases. The mean rootrot injury was 10.5 per cent for the long fallow period and 6.7 and 42.2 per cent for the two short fallow periods. Obviously, the longer fallow period has not shown a definite improvement of condition.

2. CORN, SORGHUM, AND OATS AS INTERVENING CROPS.—Of the 19 two-year rotations which include another field crop, 10 include corn, 6 include sorghum, and 3 include oats. If we disregard all other features of tillage and rotation practice, we find that the 10 rotations, including

corn, show a mean annual rootrot injury of 10.5 per cent, with a range from 1.1 to 31.9 per cent; those including sorghum, a mean of 6.5 per cent, with a range from 0.8 to 19.3 per cent; those including oats show a mean of 10.4 per cent with a range from 2.8 to 16.7 per cent. It is clear from these figures that these different crops do not effect differently the rootrot injury on succeeding crops of cotton.

TABLE III.—*Percentage of plants killed by rootrot in the various two-year rotations at the San Antonio Experiment Farm, 1912 to 1919*

Rotation and plot No.	1912	1913	1914	1915	1916	1917	1918	1919	Rotation mean.
A–4a A4–1	.....	0.1	.....	1.3	.....	4.7	.....	48.6	10.5
A–4a A4–2	0	.....	0	.....	3.2	.....	26.5	.....	
A–4d A4–7	.....	1.5	.....	14.0	.....	29.1	.....	27.5	13.0
A–4d A4–8	0	.....	0	.....	12.5	.....	19.3	.....	
A–5c1 A5–7	0.3	.....	.2	.....	13.0	.....	9.8	.....	11.7
A–5c1 A5–8	.....	2.1	.....	11.6	.....	17.3	.....	39.0	
A–5c2 A5–9	0	.....	.1	.....	18.0	.....	30.0	.....	10.4
A–5c2 A5–10	.....	.3	.....	4.3	.....	5.6	.....	24.8	
A–5d1 A5–11	.2	.....	.2	.....	22.6	.....	21.2	.....	16.7
A–5d1 A5–12	.....	1.1	.....	14.3	.....	15.0	.....	59.0	
A–5d2, A5–13	.2	.....	0	.....	43.2	.....	46.9	.....	19.3
A–5, A5–14	.....	1.2	.....	6.7	.....	20.8	.....	35.4	
A–5e1 A5–15	.3	.....	1.4	.....	14.8	.....	4.0	.....	2.8
A–5e1 A5–16	.....	.1	.....	0	.....	.2	.....	1.5	
A–5e2 A5–17	0	.....	0	.....	3.7	.....	3.4	.....	1.2
A–5e2 A5–18	.....	0	.....	.2	.....	1.6	.....	.9	
B–5a 9	.....	0	.....	0	.....	0	.....	3.3	.8
B–5a 10	0	.....	.1	.....	2.2	.....	.4	.....	
B–5b 11	.....	0	.....	.2	.....	0	.....	8.6	3.2
B–5b 12	0	.....	.1	.....	5.6	.....	11.0	.....	
B–5e 17	.....	2.2	.....	1.3	.....	4.1	.....	15.1	4.3
B–5e 18	.6	.....	.3	.....	3.0	.....	8.1	.....	
B–6a 1	.7	.....	0	.....	2.0	.....	4.9	.....	1.1
B–6a 2	.....	0	.....	0	.....	.8	.....	.2	
B–6b 3	0	.....	0	.....	1.3	.....	8.0	.....	2.1
B–6b 4	.....	.1	.....	0	.....	.8	.....	6.8	
B–6c 5	0	.....	.4	.....	.3	.....	1.7	.....	1.1
B–6c 6	.....	.6	.....	0	.....	1.9	.....	3.9	
B–6d 7	0	.....	0	.....	.0	.....	8.3	.....	1.9
B–6d 8	.....	0	.....	.2	.....	1.0	.....	5.4	
B–6e 9	0	.....	.5	.....	4.5	.....	13.8	.....	4.7
B–6e 10	.....	.2	.....	2.3	.....	3.9	.....	12.6	
B–6f 11	1.1	.....	3.5	.....	51.8	.....	6.5	.....	12.4
B–6f 12	.....	.7	.....	3.9	.....	10.5	.....	21.3	
B–6g 13	2.5	.....	12.8	.....	98.2	.....	8.5	.....	31.9
B–6g 14	.....	13.2	.....	45.7	.....	23.2	.....	51.3	
B–6h 15	2.0	.....	4.8	.....	81.7	.....	5.5	.....	26.3
B–6h 16	.....	1.6	.....	14.2	.....	36.6	.....	63.6	
B–6i 17	.3	.....	.6	.....	15.5	.....	31.4	.....	11.2
B–6i 18	.....	.6	.....	.4	.....	.3	.....	40.7	

3. EFFECT OF SUBSOILING.—Among the 20 two-year rotations there are five pairs of which the members of each pair differ from each other only in that in one case the land is plowed to the ordinary depth of about 8 inches, while in the other case the plow is followed once during the rotation with a subsoil plow which breaks the ground in the furrow

bottom to the depth of 4 to 6 inches additional. In the five rotations that are not subsoiled the mean of the mean annual rootrot injury is 14.2 per cent, ranging from 1.1 to 31.9 per cent. In the five subsoiled rotations the mean of the means is 7.4, and the range is from 1.2 to 26.3 per cent. Direct comparisons between the members of each pair shows that in only three of the five cases does subsoiling show a reduction of injury, and in only two cases is this difference sufficiently great to be significant.

4. EFFECT OF BARNYARD MANURE.—Direct comparison as to the effect of barnyard manure is possible in two pairs of the two-year rotations, in one pair of the annual or continuously cropped plots, and in one pair of the three-year rotations discussed below, making four in all. In those four cases the mean of the mean annual injury on the unmanured plots is 13.7 per cent, ranging from 1.1 to 42.2 per cent, while for the manured plots the mean is 9.4 per cent and the range from 1.1 to 19.3 per cent. Direct comparisons between the members of each pair shows that in two cases they have the same annual means, while in one case the manured rotation has the higher mean and in the other case the lower mean. From this it must be concluded that the use of farmyard manure has not materially reduced the injury from rootrot.

5. EFFECT OF GREEN MANURE CROPS.—The effect of a green manure crop, either cowpeas or field peas, may be compared in four pairs of rotations of which two are in the two-year group, one in the continuous-cropping group, and one in the three-year group. The mean of the annual means of the four rotations which do not have green manure crops is 14 per cent of rootrot injury, ranging from 1.1 to 42.2 per cent, while the mean for the four in which green manure is used is 10.8 per cent, with the range from 1.9 to 18.5 per cent. These differences are clearly not significant, and the conclusion must be that a green manure crop is not an effective remedy for cotton rootrot.

In addition to the foregoing simple and direct comparisons between several groups of rotation pairs, it is possible to compare the effect of combinations of different treatments against the control rotations. For example, rotation B–6a is a simple corn-cotton rotation, the land being plowed immediately after each crop and kept fallow and in good tilth until the next crop is planted. Rotation B–6b is the same, except that the land is subsoiled after the corn crop. In rotation B–6c the land is manured after the corn crop. In rotation B–6d the land is manured after the corn crop and planted to field peas, which are plowed under preceding the corn crop. In rotation B–6e the land is manured, planted to field peas, and plowed and subsoiled during the period between the corn and cotton crops. These five rotations show progressive combinations of treatments that are often recommended for controlling cotton rootrot. Reference to Table III shows that the mean annual percentage of rootrot injury was much the same in all these rotations, though slightly higher in the

last-named, in which all of the presumably beneficial treatments were combined.

One other feature of these two-year rotations remains to be considered. It is a group in which the land is spring plowed or disked immediately preceding the cotton crop. The four rotations included in this group lie in the south half of series B–6 and have all shown serious injury from rootrot. In the first of these the land is plowed only once in two years, and that immediately following the cotton crop. After the corn crop the land is disked and kept fallow by shallow tillage until the cotton crop is planted. The mean annual rootrot injury has been 12.4 per cent. In the next two rotations the land, though plowed twice in two years, is left undisturbed after the corn crop until just before planting time, when it is plowed, and one of the rotations is also subsoiled at the time of plowing. The rootrot injury on these rotations has been 26.3 per cent for the subsoiled rotation and 31.9 per cent for the one that is not subsoiled. In the fourth rotation in this group the land is disked after the corn crop and sown to rye for green manure which is plowed under in the spring just before the cotton is planted. The annual rootrot injury in this rotation is 11.2 per cent. While it seems inadvisable to make direct comparisons between these rotations and other cotton-corn rotations such as B–6a, because of the difference in field location, it is clear that none of these treatments has checked rootrot injury.

As a summary of these results from the two-year rotations, it may be said that the extent of rootrot injury appears not to have been influenced either by different crop sequences or by different tillage methods or by the use of barnyard manure or green manure crops.

THREE-YEAR AND FOUR-YEAR ROTATIONS

On the black lands of Texas where cotton is the leading field crop it is hardly practicable to consider the use of a rotation which does not involve cotton as frequently as every third year, or at most, every fourth year. If a period of two years or three years without cotton is not sufficient to secure a rotation effect, then it is clear that rotation effect must be dispensed with. As indicated above, the San Antonio rotation experiment includes four three-year rotations and two four-year rotations. The detailed record of rootrot injury in these six rotations is shown in Table IV. Two of the three-year rotations and both of the four-year rotations were started in 1913.

While four of the six rotations included in Table IV show very slight rootrot injury, the other two—that is, one three-year and one four-year—show an extent of injury comparable with the plots cropped to cotton continuously. In other words, these results do not justify the hope that a relatively long period without cotton leaves the land less likely to show rootrot injury when cotton is again planted. It is true that these longer rotations differ from each other in certain respects, but these differences

are of the same sort that in the shorter rotations were found to be without effect on the extent of rootrot injury. Thus, rotation B–4a, which shows a mean annual loss of only 0.6 per cent, differs from rotation B–4b with a annual loss of 6.9 per cent only in having a year of Sudan grass instead of oats followed by field peas. Likewise, in the four-year rotation one of these, A–4f, with an annual loss of 0.4 per cent, differs from the other, having an annual loss of 15.0 per cent, only in that the field peas are cut for hay instead of being plowed under. These differences of treatment can hardly be regarded as sufficient to account for the striking differences in the extent of rootrot injury.

Having in view the whole of the results from these rotation experiments, one is forced to the conclusion that the control of rootrot is not to be found through any ordinary system of crop rotation or of tillage methods.

TABLE IV.—*Percentage of plants killed by rootrot in the various three-year and four-year rotations at the San Antonio Experiment Farm, 1912–1919*

THREE-YEAR ROTATION

Rotation and plot No.	1912	1913	1914	1915	1916	1917	1918	1919	Rotation mean.
A–5a 1	.....	0	.....	.....	2.0	.....	.....	4.7	1.3
A–5a 2	0	.....	.....	0	.....	.....	0	.....	
A–5a 3	.....	.....	2.0	.....	.....	1.8	.....	.....	
A–5b 4	.....	0	.....	.....	0	.....	.....	4.2	1.2
A–5b 5	0	.....	.....	0	.....	.....	2.5	.....	
A–5b 6	.....	.....	(a)	.....	.....	1.6	.....	.....	
B–4a 14	.....	.....	(a)	.....	.....	0	.....	.....	.6
B–4a 15	.....	0	.....	.....	0	.....	.....	1.8	
B–4a 16	.....	.....	.....	.9	.....	.....	.7	.....	
B–4b 17	.....	.....	(a)	.....	.....	2.6	.....	.....	6.9
B–4b 18	.....	.2	.....	.....	9.3	.....	.....	26.5	
B–4b 19	.....	.....	.....	1.4	.....	.....	1.5	.....	

FOUR-YEAR ROTATION

A–4f 11	.....	.....	.....	.....	1.7	.....	.....	.....	.4
A–4f 12	.....	.....	.....	0	.....	.....	.....	.7	
A–4f 13	.....	.....	0	.....	.....	.....	.1	.....	
A–4f 14	.....	.4	.....	.....	.....	.1	.....	.....	
A–4g 15	.....	.....	.....	.....	25.6	.....	.....	.....	15.0
A–4g 16	.....	.....	.....	18.5	.....	.....	.....	51.9	
A–4g 17	.....	.....	1.5	.....	.....	.....	.5	.....	
A–4g 18	.....	3.8	.....	.....	.....	2.9	.....	.....	

[a] No record.

LIFE HISTORY OF RECURVARIA MILLERI, THE LODGEPOLE PINE NEEDLE-MINER, IN THE YOSEMITE NATIONAL PARK, CALIFORNIA

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INTRODUCTION

The lodgepole pine needle-miner (*Recurvaria milleri* Busck) infests the needles of the host tree, *Pinus murrayana* Oreg. Com., causing them to fade and later fall from the trees. The result is defoliation in the infested areas. Stands of timber affected by the needle-miner can not be distinguished superficially by the untrained woodsman from those infested by bark-beetles of the genus Dendroctonus. To prevent needless waste of timber and money, therefore, it is very desirable that control operations against barkbeetles, namely, the felling of the trees, should not be undertaken where only the less serious, and usually not fatal, needle-miner infestation occurs.

The only serious epidemics of the needle-miner which have been recorded occur in the lodgepole pine stands of the Yosemite National Park of California. The studies presented in this paper were made in these epidemic areas during the summer seasons of 1917, 1918, and 1919. The investigations were confined exclusively to the Yosemite National Park, and the studies presented apply specifically to the insect in this locality only.

HISTORICAL REVIEW

In 1903 it was reported to the Bureau of Entomology through the Secretary of the Interior that large areas of lodgepole pine in the Yosemite Park were affected by a leaf-mining moth. In May, 1904, Dr. A. D. Hopkins visited the Yosemite Park, planning to investigate the conditions which had been reported, but was unable to reach the lodgepole pine areas, as all trails leading into the region were still closed by heavy snows. In 1906 Mr. H. E. Burke spent the month of July in the Tenaya Basin and Tuolumne Meadows, but found no evidence of the needle-miner in that vicinity, although a considerable amount of timber was being killed by the mountain-pine beetle (*Dendroctonus monticolae* Hopkins). Later, in 1907, Prof. J. H. Comstock reported its presence in the park to Dr. L. O. Howard, stating that he saw large areas of tamarack pine (as lodgepole pine is called locally) infested by the moths.

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During the summer of 1911 Forest Pathologist Dr. E. P. Meinecke made a reconnaissance through the Tenaya Basin and Tuolumne Meadows and reported widespread infestation by a needle-miner and a heavy flight of moths which occurred in July and August of that year.

In October, 1912, Mr. J. M. Miller made a trip into the Tenaya Basin and on into the Tuolumne Meadows and found that the barkbeetle infestation reported by Mr. Burke had spread and was threatening the lodgepole pine stands in both the Tenaya and Cathedral Basins. At the same time the infestation of the lodgepole pine needle-miner was conspicuous in both watersheds. At that time the insect was in the larval stage in the needles of the host, having mined such a high percentage of them that the foliage cast of the entire forest presented a dull brownish color. The general effect was suggestive of the scorching resulting from fire. Subsequent examinations by Mr. Miller in 1913 and 1914 indicated that the infestation of the insect was distributed throughout about 30,000 acres in the National Park, extending through the main Tuolumne Watershed and the upper basin of Tenaya Creek, a tributary of the Merced. It was limited, however, to lodgepole pine growing between the elevations of 7,000 and 9,000 feet. Where the infestation had been prevalent for several years, the stands were usually defoliated to a considerable degree. Adult specimens collected by Mr. Miller in 1913 were later described as a new species, *Recurvaria milleri*, by Mr. August Busck.[1]

The writer was assigned in 1917 to a special study of the insect by his immediate superior, Mr. Miller.[2] The program of the work as outlined at that time consisted of field studies and experiments to be conducted throughout the period of activity of the insect in the infested areas during the spring and summer of 1917, 1918, and 1919. This original outline was not changed, and the investigations were carried out in connection with the studies of a barkbeetle which infests the host tree in the same locality.

During the early summer of 1918 Mr. Carl Heinrich, specialist in forest Lepidoptera of the Bureau of Entomology, accompanied the writer on a field trip into the infested areas.[3]

CONDITION OF INFESTED STANDS

Where the needle-miner has been prevalent, the first noticeable result, aside from the brownish cast of the foliage caused by the color of the affected needles, is a distinct phase of defoliation. This is caused by the falling of a very high percentage of the needles which have been attacked. After several years of repeated defoliations a noticeable dying of the crowns and ends of branches, a stunting of growth, and a general decadent

[1] BUSCK, August. DESCRIPTIONS OF NEW MICROLEPIDOPTERA OF FOREST TREES. *In* Proc. Ent. Soc. Wash., v. 16, no. 4, p. 143–150, pl. 7–8. 1914.

[2] Studies and records made by Mr. Miller in 1913 and 1914 have been freely used by the writer as a basis for his investigations, and acknowledgment is made of the value of these first records.

[3] Suggestions made by Mr. Carl Heinrich at the time of this trip and subsequent assistance in the determination of material sent to him have been of great help to the writer.

appearance of the trees becomes apparent (Pl. 30). It has been noted that this latter damage is most severe and conspicuous in mature or overmature stands, such as are found in the Tenaya and Cathedral Basins. There is a striking contrast in the degree of damage in different localities of the infested area. In the northern part of the Tuolumne Basin, in the vicinity of Kerrick Canyon, the damage is much less severe than in the Tenaya and Cathedral Basins, although the infestation has been present fully as long in the northern locality as in the southern. Dying tops and limbs would, of course, result from pathological conditions produced by other causes than the needle-miner. No disturbances aside from the needle-miner have been noted; but the pathological aspects of the problem have not been thoroughly studied.

It has also been noted that the degree of damage varies greatly according to the site of the host tree. Trees standing on rocky, exposed sites, under unfavorable soil and moisture conditions, suffer much more severely than those standing on meadows where there is abundant soil and moisture.

Much of the timber defoliated by the needle-miner has been attacked and killed by an entirely different insect, the mountain-pine beetle (*Dendroctonus monticolae*). No direct biological association, however, exists between the needle-miner and the barkbeetle, although the badly defoliated trees are apparently so weakened that they offer little, if any, resistance to the attacks of the beetles.

Final and complete killing of the trees as a result of the needle-miner damage alone has not been recorded, although some of the more severely affected have reached a condition that offers little hope for their ultimate recovery. The insidious character of the needle-miner damage is not readily apparent in areas of recent invasion, but is very noticeable in stands where the infestation has persisted for a number of years. This will be readily understood by reference to the diagrams (fig. 1–3), which show that the attacks upon the needles occur in such manner as to allow the trees a breathing spell sufficient to maintain their vitality for a few years after the initial attacks.

LIFE CYCLE

The length of the life cycle is 25 months and covers a period of 1 year and parts of 2 other years. The individual broods hibernate during two winter periods of approximately 6 months each. The feeding periods of the larvæ occur during parts of 3 years, aggregating a total period of 11 months. Flight occurs every alternate year, as in 1913, 1915, 1917, and 1919. During the alternate years from flight the broods are found in the larval stage in the needles of the current or previous year's growth.

Emergence and flight of the adults occur during the period from July 1 to August 31. For illustration of life cycle see figure 1. Some few individuals may be seen earlier or later than these dates, but they represent only advanced individuals and stragglers. During the period of

maximum flight thousands of adults may be seen on the wing and crawling over the foliage of the host and other trees. Many of them fall into the streams and on the numerous small lakes and perish; thousands may be seen floating on the surface of the water. During this period the flying moths become a pest to campers in the locality, as they invade the tents and fall into the cooking utensils and provisions.

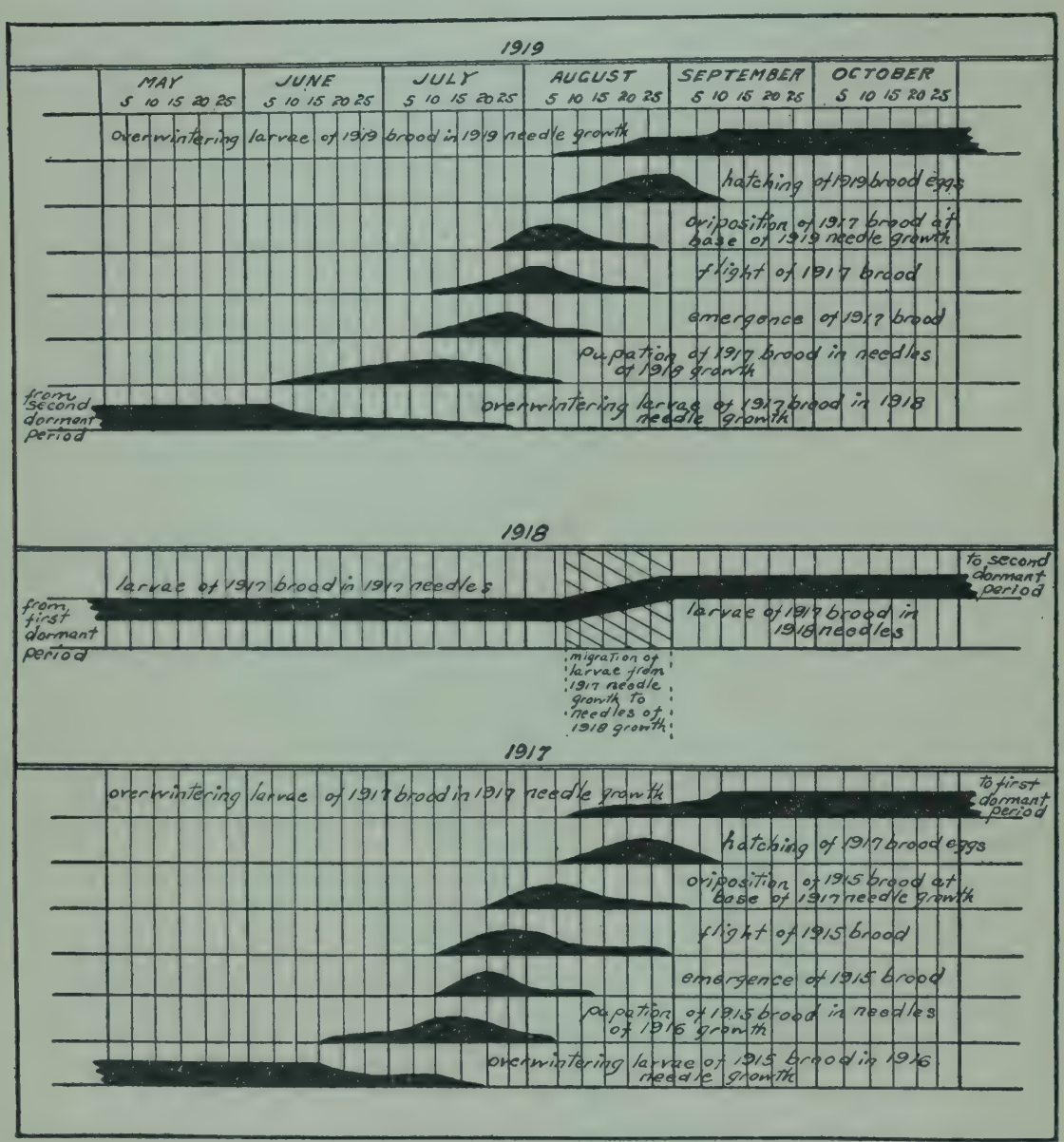

1919
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
overwintering larvae of 1919 brood in 1919 needle growth
hatching of 1919 brood eggs
oviposition of 1917 brood at base of 1919 needle growth
flight of 1917 brood
emergence of 1917 brood
pupation of 1917 brood in needles of 1919 growth
overwintering larvae of 1917 brood in 1918 needle growth
from second dormant period
1918
larvae of 1917 brood in 1917 needles
from first dormant period
larvae of 1917 brood in 1918 needles
to second dormant period
migration of larvae from 1917 needle growth to needles of 1918 growth
1917
overwintering larvae of 1917 brood in 1917 needle growth
to first dormant period
hatching of 1917 brood eggs
oviposition of 1915 brood at base of 1917 needle growth
flight of 1915 brood
emergence of 1915 brood
pupation of 1915 brood in needles of 1916 growth
overwintering larvae of 1915 brood in 1916 needle growth

FIG. 1.—Diagram illustrating the life history of *Recurvaria milleri* at Lake Tenaya, Yosemite National Park, Calif.

The eggs are deposited on and under the needle sheaths at the base of the needles. Occasionally eggs are found on the terminal shoots near the base of the needles. Larvæ begin to hatch about August 5 and continue hatching until about September 10. Attack by the young larvæ on the tender needles of the current year's growth begins soon after hatching. The young larvæ invariably attack the new needles by boring in near the outer ends, and they always work toward the petiole, never in the opposite direction. This peculiarity of habit permits of a

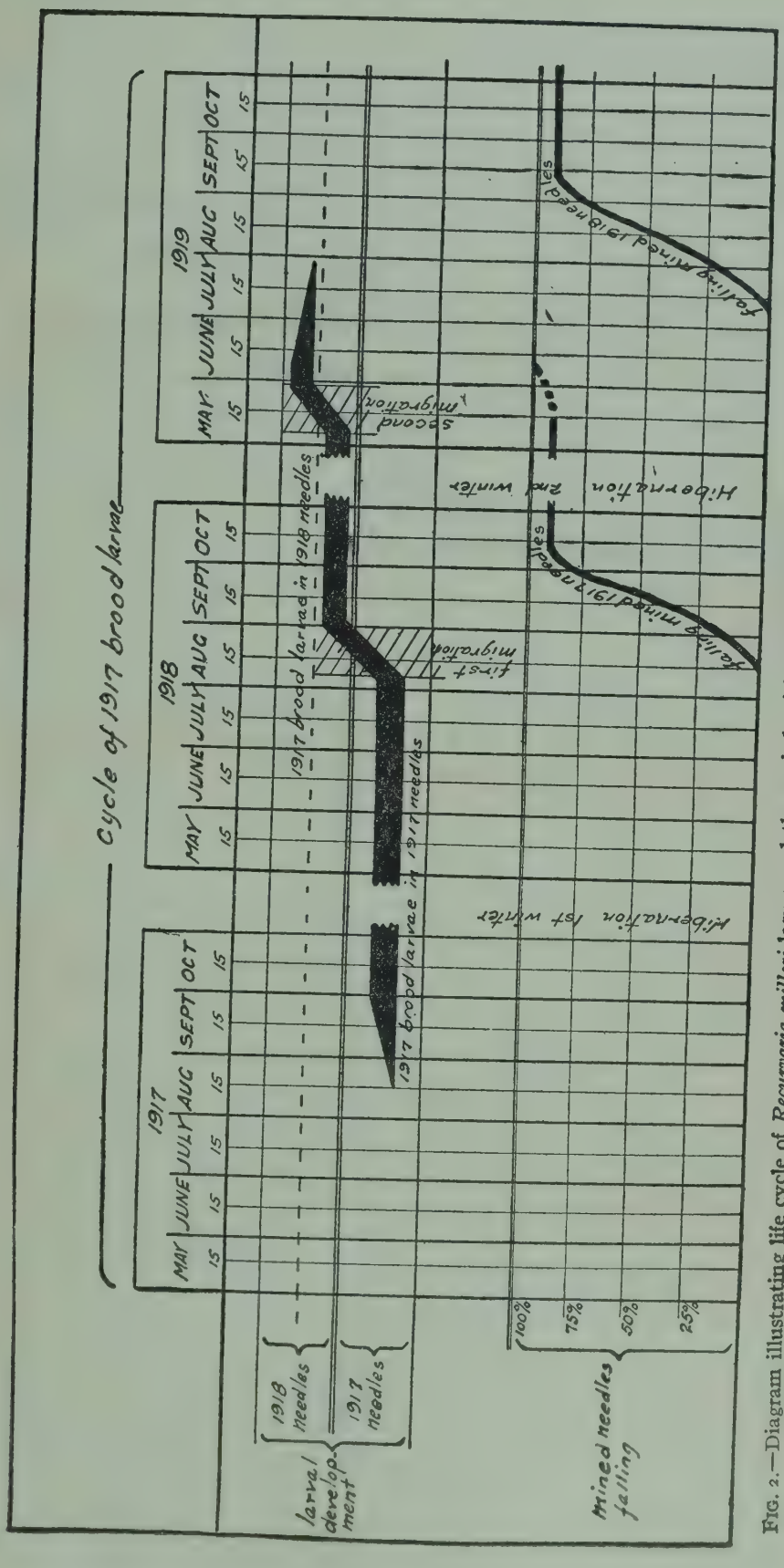


FIG. 2.—Diagram illustrating life cycle of *Recurvaria milleri* larvæ and the periods of larval migrations, as well as the periods of falling of the mined and abandoned needles.

longer life for the attacked needles and insures fresh material for the larvæ to feed upon. By the latter part of October the larvæ have grown to be approximately 2 mm. long and have mined about one-third the outer length of the infested needles.

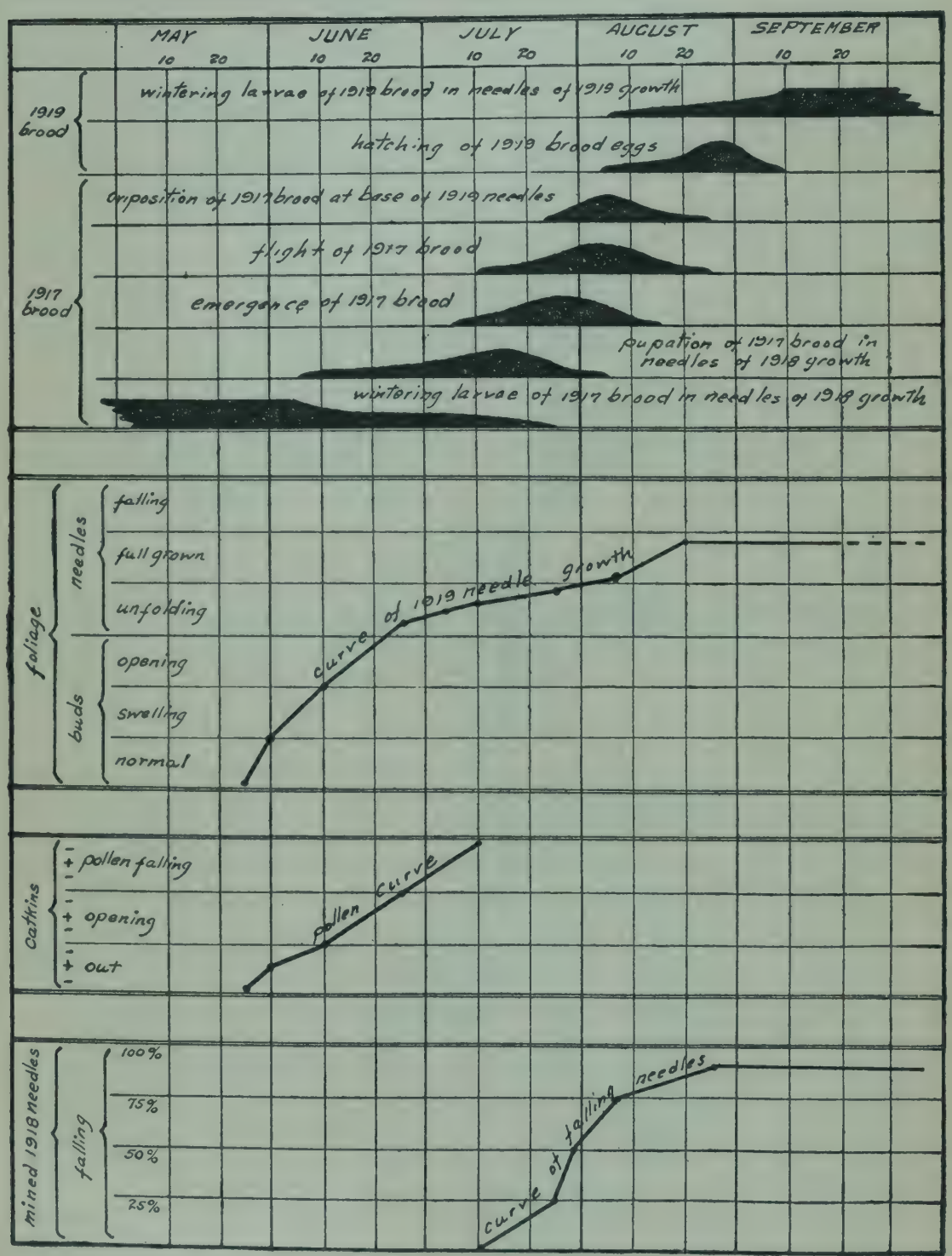


FIG. 3.—Diagram illustrating seasonal activity of *Recurvaria milleri* and the corresponding phenological phenomena of the host plant.

Owing to the high elevations, above 7,000 feet, of the infested areas, winter conditions set in during the latter part of October and insect activity ceases until the following spring. During the winter of the first season the insect hibernates as immature larvæ in the partially mined needles.

Activity is resumed in early May of the next spring, and the brood develops as larvæ throughout the growing period of the second season. By the middle of July the larvæ have mined two-thirds the length of the infested needles attacked during late summer of the previous year and are about half-grown, or nearly 4 mm. long.

Beginning about August 5 of this season, or when the new needles of this year's growth are nearly full-grown, the larvæ begin to leave the mined needles in which the previous winter was passed and migrate to these new needles. The migration covers the period from August 5 to September 1, when practically all the brood is in the new needles (fig. 2, 3). During this period the migrating larvæ are exposed and may be seen crawling about on the needles and twigs. Undoubtedly a high mortality occurs during the migration, but no statistics are available to show this. By the first of September the larvæ are well established in the new needles of the current year's growth which are attacked near the outer ends. These new and tender needles are mined very rapidly so that by September 15 one-half the length of each infested needle has been eaten.

Toward the latter part of October and the arrival of winter conditions the brood again ceases feeding and becomes dormant, passing the second winter as nearly mature larvæ in the first-attacked needles of the second season's growth.

Activity is again resumed the following spring in early May. During this spring-feeding period the larvæ complete the mining of the needles in which the brood hibernated. These mined needles are then abandoned and the larvæ attack green needles of the same growth, which by the last of May are from one-half to three-fourths mined; the larvæ, now being full-grown, cease feeding and prepare the larval tunnels for pupation in the needles mined last. These tunnels are slightly lined with silk and have emergence tubes leading to the covered exit holes near the tips of the needles. By the close of the second spring-feeding period each larva has mined an approximate average of two needles of the preceding year's growth. Thus, each larva mines three needles during its life cycle of 23 months—one during the first season of attack and one after each of the two migrations (fig. 2). From this it is obvious that of the crop of needles which develops after the season of initial attack twice as many needles are mined as of the crop which receives the initial attack. This accounts for the more conspicuous color phase and defoliation which are apparent every alternate year (Pl. 29, D).

Pupation begins about June 10 and is continued until August 5. Pupæ form in the mined needles with the heads toward the tips. Emergence of the adults begins about July 1. Soon after the mined needles are abandoned by the migrating larvæ or the emerging adults, the needles begin to fall from the trees (fig. 2, 3).

THE MOTH

DESCRIPTION

The adult is a small, grayish moth with a wing expanse of 12 to 15 mm. The head, fore wings, and thorax are of a silvery gray color irregularly marked with black. The antennæ are white annulated with black. The face is white. The hind wings are white dusted with fuscous; the cilia slightly ocherous. The abdomen is silvery white. The legs are white barred with black. The females are slightly larger than the males. (Pl. 29, A.)

EMERGENCE, LONGEVITY, AND HABITS OF FLIGHT

The moths emerge every alternate year, as in 1913, 1915, 1917, and 1919. First adults appear in the field between July 5 and 10, and emergence is continued until about August 15, reaching a maximum about July 25. The average length of life of the adults, as appears from data obtained on adults reared in field cages, is approximately 14 days. All observations indicate that the moths are most active during the warmer part of the day; myriads may be seen flying during the maximum period of flight and active on the foliage of the host on warm days between 10 a. m. and 4 p. m. After 4 p. m. at these high altitudes the temperature drops considerably and the moths cease flying and are not so active on the foliage. On cloudy days the moths hide away among the foliage and in the crevices of the bark of the tree trunks and fly out only when disturbed.

MATING AND EGG LAYING

Copulation was observed in the field on one occasion only. Numerous observations were made in the field and on moths kept captive in a cage to determine the approximate period of mating, but this one instance is all that was recorded. This occurred about 11 a. m., July 25, 1919. When first noted the pair was attached and quietly resting on a small lodgepole pine branch a few inches from its outer end.

Oviposition was observed once only and in the field at 1 p. m., July 27, 1919. When first noted, the moth was in position to oviposit at the base of the 1919 needle growth on the tip of a small twig about 5 feet above the ground. It was facing the tips of the young needles and had the ovipositor inserted between and under the needle sheaths and against the branchlet. This appears to be the normal position, as practically all the eggs that were found in the field were located under the needle sheaths or on the surface of the twigs near the base of the 1919 needle growth. (Pl. 29, B.) After this moth had been under observation for five minutes she was collected and it was found that she had deposited three eggs in this position. Later the moth was dissected and nine more eggs were secured. The foregoing record of oviposition was made

during the period of maximum flight of the adults. On numerous other occasions moths were observed running actively over the foliage, occasionally stopping and pressing the tip of the abdomen against the base of the needles as though attempting to oviposit. Freshly laid eggs were found in the field during the latter part of July and throughout August, 1919. (Table I.)

TABLE I.—*Period of egg laying of Recurvaria milleri*

Date of observation.	Number of eggs in record.
1919.	
July 24.....	3, at base of needles.
27.....	7, at base of needles.
30.....	8, at base of needles.
Aug. 4.....	10, at base of needles.
8.....	5, at base of needles.
20.....	8, at base of needles.
25.....	3, at base of needles.

It was not determined whether one individual female deposits eggs in more than one place or not; neither was the total number of eggs deposited by individual females ascertained. Clutches of eggs found in the field averaged from five to six in number. (Tables I and II.)

TABLE II.—*Number of eggs of Recurvaria milleri found in clutches in the field*

Clutch No.	Number of eggs.
1	3
2	7
3	8
4	1
5	10
6	3
7	5
8	8
9	1
10	3
Total.....	49

THE EGG

DESCRIPTION

The normally deposited egg is oblong oval, rugose, and translucent, with iridescent surface reflections. The average size is 0.3 by 0.5 mm.

WHERE DEPOSITED

The eggs are usually deposited at the base of the current year's growth of needles and are concealed under the needle scales. Occasionally they

are found on the surface of the terminal twigs, but always close to the late needle growth. Wherever found they are not securely attached. (Pl. 29, B.)

PERIOD OF INCUBATION

Incubation lasts approximately from 12 to 14 days. The maximum time required for the eggs to develop, as recorded in the season of 1919, was 14 days, the minimum 12 days, and the average 13 days. Records on the incubation of eggs for the season of 1919 are given in Table III.

TABLE III.—*Incubation period of eggs of Recurvaria milleri, season of 1919*

Number of eggs in record.	Date of deposition.	Date of hatching.	Incubation period.
			Days.
5	July 27	Aug. 8	12
3	27	9	13
1	30	13	13
4	30	14	14

THE LARVA

DESCRIPTION

The mature larva is roughly cylindrical; its average length is 8 mm., or from seven to nine times the width. The color varies from light lemon yellow to deep orange, with an indistinct darker red patch along the dorsal median line. The head is black with dark brown thoracic shield, and the anal plate is lighter brown. The prolegs are on the sixth, seventh, eighth, and ninth abdominal segments.

HABITS OF LARVÆ

The larvæ feed on the interior of the needles by boring in near the outer ends and working toward the bases, constructing longitudinal mines which vary in length from one-third to the entire length of the needles (Pl. 29, C). The frass and excrement are ejected through the entrance hole as fast as accumulated. The larvæ are very active; when disturbed they retreat through the needle mines, backing out through the entrance holes, and occasionally letting themselves down by means of a thread spun from the silk glands.

The first stages of the larvæ are passed in the young needles of the current year's growth. The first winter is also passed in these needles. When the needles of the next crop arrive the following summer the larvæ migrate to them and feed until the dormant period of the second winter begins; the second hibernation occurs in these needles. When activity is resumed the second spring the larvæ complete the mining of the needles in which the winter was passed and enter fresh needles of the

same growth. When these are partially or completely mined the now mature larvæ prepare pupal cells in the last-mined needles and envelop themselves in thin silk. During its life each larva mines an average of three needles. (Fig. 1.)

LENGTH OF LARVAL STAGE

The maximum length of the larval stage is 23 months, or from August 15 of the first year to July 1 of the third year (Table IV).

TABLE IV.—*Duration of the larval stage of Recurvaria milleri*

Date of observation.	Development and activity of larvæ.
Aug. 15, 1917, to June 1, 1918.	No observations were recorded for this period.[a]
June 4, 1918	Larvæ 3 mm. long; feeding in needles of 1917 growth.
June 13, 1918	Larvæ 3.5 mm. long; feeding in needles of 1917 growth.
June 22, 1918	Do.
July 13, 1918	Larvæ 4 mm. long; feeding in needles of 1917 growth.
July 20, 1918	Larvæ 5 mm. long; feeding in needles of 1917 growth.
Aug. 5, 1918	Larvæ in mined needles of 1917 growth and entering unmined needles of 1918 growth.
Aug. 12, 1918	Larvæ in mined needles of 1917 growth and entering unmined needles of 1918 growth; many larvæ in migration exposed on foliage.
Aug. 23, 1918	Larvæ in mined needles of 1917 growth and entering unmined needles of 1918 growth; only a few individuals remaining in mined needles of 1917 growth.
Sept. 4, 1918	Larvæ 5.5 mm. long; all in needles of 1918 growth.
Sept. 15, 1918	Do.
Sept. 16, 1918, to May 24, 1919.	No observations were recorded for this period.[a]
May 24, 1919	Larvæ 6 to 7 mm. long; in mined needles of 1918 growth.
	Larvæ migrating from mined 1918 needles to unmined needles of same growth.
June 1, 1919	Do.
June 10, 1919	Larvæ 7 to 8 mm. long; in freshly mined needles of 1918 growth.
June 25, 1919	Prepupal larvæ and pupæ in mined needles of 1918 growth.
July 10, 1919	Do.
July 25, 1919	Do.

[a] The absence of records for these periods is due to the author's absence from the park.

THE PUPA

DESCRIPTION

The pupæ are dark brown to black in color and are approximately 1.2 mm. in diameter by 6 mm. long. They are found in thin silk cocoons in the last needles mined by the larvæ. (Pl. 29, C.)

LENGTH OF PUPAL STAGE

The maximum pupal stage lasts from June 20 to July 20, or 30 days.

HOST PLANT

These studies were confined exclusively to the Yosemite National Park, Calif., wherein the insect was found attacking only one host tree, the lodgepole pine (*Pinus murrayana*).

NATURAL ENEMIES

In connection with the work on this species, the following 10 species of hymenopterous parasites have been reared from prepupal larvæ:

Apanteles n. sp.	Det. C. N. Muesebeck
Angitia sp.	Det. R. A. Cushman
Aethecerus n. sp.	Det. R. A. Cushman
Scambus sp.	Det. R. A. Cushman
Epiurus sp.	Det. R. A. Cushman
Euteles n. sp.	Det. S. A. Rohwer
Habrocytus n. sp.	Det. S. A. Rohwer
Copidosoma sp.	Det. A. B. Gahan
Elachertus sp.	Det. A. B. Gahan
Eulophid	Det. A. B. Gahan

Of these parasites the Copidosoma and the Euteles are most abundant. In 1919 the writer obtained only the following: *Euteles* n. sp., *Copidosoma* sp., *Epiurus* sp., *Aethecerus* n. sp., *Apanteles* n. sp., *Habrocytus* n. sp., and the eulophid. In this year 234 of the 1,886 host larvæ examined were parasitized; thus the seven species listed above produced in this year a parasitism of approximately 12 per cent.

In addition to the foregoing insect enemies there is a small bird known locally as the "pine siskin" that in a few instances has been observed feeding on the adults during the flight period.

LARVAL MORTALITY

During the studies in the spring of 1919 a number of mature larvæ were collected to determine the relative mortality in this stage. Mortality during this stage only was studied, as it was practically impossible to obtain field data of this character for any other stage. A total of 1,886 larvæ were examined; of this number 178, or 9 per cent, were dead and more or less withered. The cause of this mortality was not determined, though it is probably a bacterial disease.

DISTRIBUTION

The distribution of the lodgepole pine needle-miner in the Yosemite National Park is confined to the lodgepole pine stands in the higher elevations north of the Merced Canyon. The extent of this distribution is shown on the map (fig. 4).

One striking feature of its distribution is its occurrence only in distinct zones or areas of epidemic infestation. Within these areas the infestation is abundant, involving every tree with resultant severe damage to the entire stand. Outside of the infested areas the insect does not occur in the larval stage at all, or in such minor degree that no trace of it can be found. Only along the borders of the infested areas have trees been found which contain only a low percentage of affected needles.

These areas of epidemic infestations occur only in belts between two fairly definite contours of elevation. The larval infestation has not

been found below 7,000 feet elevation and rarely above 9,000 feet, although the lodgepole pine stands extend both above and below these contours.

The reasons for these distinct limits to the distribution of the insect have not been determined. The moths in flight have been recorded in

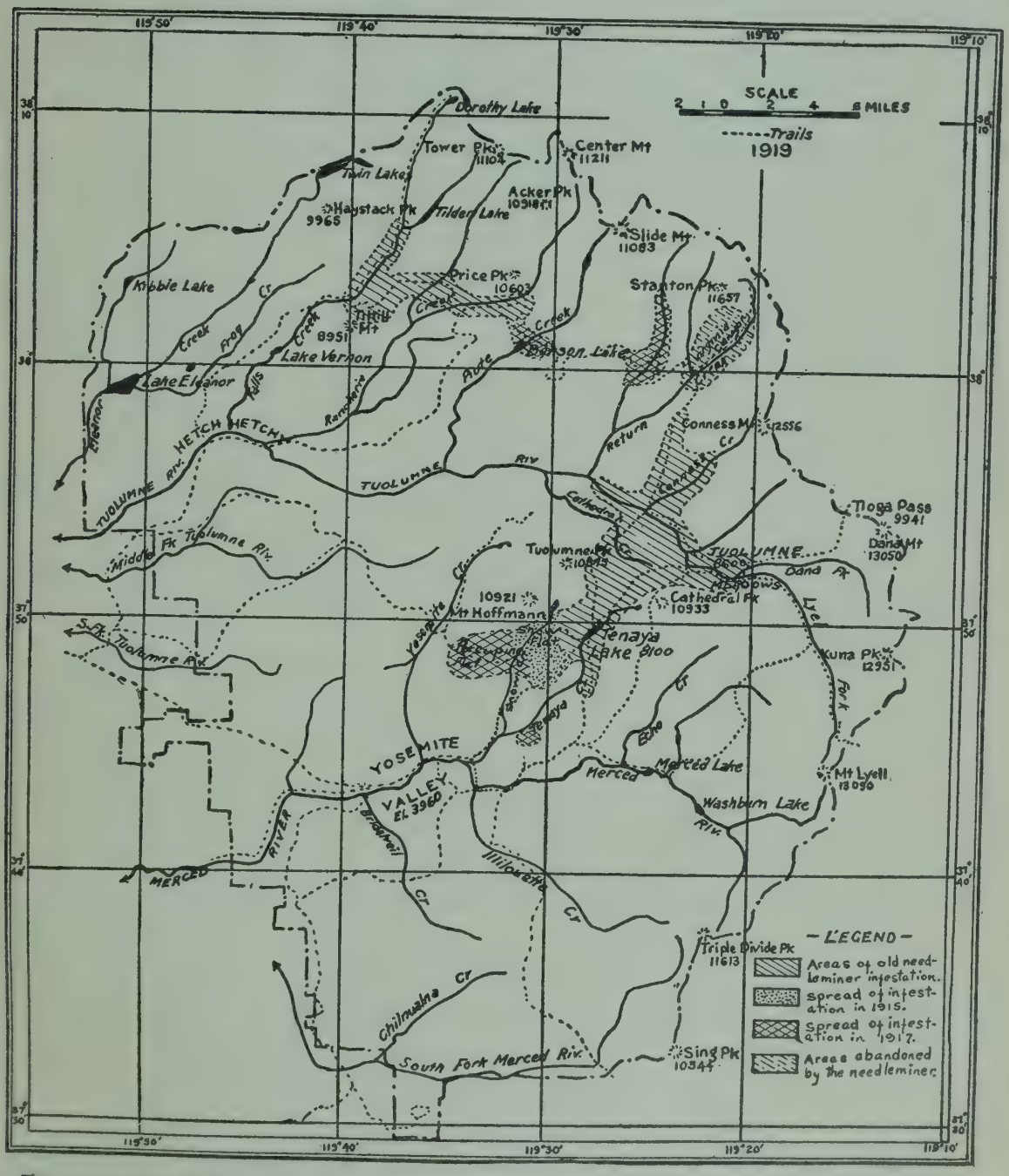


FIG. 4.—Map of the Yosemite National Park, Calif., showing range of distribution and areas infested with *Recurvaria milleri*, 1919.

localities many miles outside of the infested areas; in one instance in the Yosemite Valley at an elevation of 4,000 feet, 6 miles distant from the nearest infestation, and on Cathedral Peak, at an elevation of 10,000 feet, moths were abundant 3 miles distant from the nearest infestation. No trace of larval infestation has ever been found in either locality.

In 1919 the infested areas were determined as follows: South of the Tuolumne River in Tenaya and Cathedral Creek Basins, Snow Flat and Porcupine Flat, and Ten Mile Meadows; on the headwaters of the Tuolumne River throughout the Tuolumne Meadows as far east as Lambert Dome and the terminus of the Lyell Fork Canyon; down the Tuolumne River to and throughout Conness and Alkali Creek Basins and Glen Aulin. The infested areas north of the Tuolumne Canyon occur in the Matterhorn Canyon, around Benson Lake, and throughout the canyons of Kerrick, Stubblefield, and Jack Main. The range of the infestation covers an approximate total area of 60 square miles, or 39,000 acres.

The records now available indicate an advance of the infestation toward the southwest. In 1906 no evidence of the needle-miner was found in Tenaya Basin or the Tuolumne Meadows. In 1911 the infestation was prevalent and conspicuous in both these areas. In 1912 it had not advanced beyond the divide between Tenaya and Snow Flat, but in 1917 infestation was very heavy throughout this latter area, and in 1919 it was conspicuous in Porcupine Flat still farther southwest. The limits of the host type are reached in Porcupine Flat, so that further advance to the west will necessitate a migration of 6 miles over a high ridge to the lodgepole pine stands in Yosemite Creek Basin.

Records show that the invasion of a new area occurs suddenly, the infestation reaching an epidemic condition in one year, with resultant very heavy defoliation from its inception.

Old centers of infestation are indicated in the stands in Jack Main and Matterhorn Canyons. The greater portion of these stands is now dead, having been killed by the mountain-pine beetle years ago, but all indications point to the fact that the needle-miner was present in these areas prior to the advent of the beetle. The needle-miner is still present in both these canyons, infesting the remaining trees.

In Virginia Canyon, north of the Tuolumne Meadows, the needle-miner was present in 1911, as shown by the mined needles found under the trees in 1913 and the absence from the twigs of a heavy percentage of the 1910 needle growth; but for some reason that has not been determined the moths abandoned the area in that year, and no trace of existing infestation has since been found. This is the only area now free of infestation in which previous infestation has been known to exist.

Heavy stands of lodgepole pine occur in the Illilouette Basin and in other areas south of the Merced Canyon. These are the only large stands of lodgepole pine left within the park that have not been invaded by the needle-miner, although they are situated in the same zones of elevation as are the heavily infested areas around Tenaya. Invasion of these stands by the needle-miner may occur at any time; in fact, the infestation is drifting in this direction, as is indicated by a small area of infestation which appeared in 1919 on the slopes of Clouds Rest just across the Merced River from the Illilouette Basin.

POSSIBILITIES OF CONTROL

It is evident that the insects and other natural enemies of the moth have not been sufficient to maintain control on the greater part of the area involved in the epidemic. Artificial measures are at once suggested by the information now available relating to the various stages of the insect's life history.

The use of sprays, which is usually resorted to for the control of infestation of this character, does not appear practicable for application throughout extensive forest areas where every tree is infested, because this method to be successful requires very thorough and frequent applications. Because of the peculiar life history of the species, however, contact sprays would be more valuable than stomach poisons since, during the egg and initial larval stage and before the young larvæ have entered the tips of the needles, both eggs and larvæ are exposed and contact sprays would reach them. Stomach poisons would be effective only when the larvæ are eating their way into the needles.

The infestation could also be attacked by felling the infested trees. During the feeding periods of the larvæ the felling of the trees would cause the death of the needles and the larvæ thus deprived of their food supply would soon succumb. This method, however, would call for the felling of every infested tree in the entire stands involved in the epidemics. For this reason the method is impracticable and can not be considered except in the case of a few trees which might threaten a non-infested stand. Such situations, however, have not been observed in this epidemic.

On the whole, it is doubtful if costs of applying any method of control against the moth would be warranted by the present value of the lodgepole pine stands in the Yosemite National Park. This species has little commercial value, and even for park purposes it is not nearly so desirable as other species.

These silvicultural features should be considered in any scheme of forest protection for the Yosemite National Park. In many sites where the mature lodgepole pine has been killed out by barkbeetle epidemics following in the wake of the needle-miner invasions, mountain hemlock and fir are reproducing to a most encouraging extent. These latter species are more desirable for park purposes in many ways and are not subject to the insect epidemics which are killing the lodgepole pine. It is the opinion of the writer that the great amount of funds which would be required for the protection of the lodgepole pine could be spent to better advantage to encourage the reproduction of more desirable species.

PLATE 29

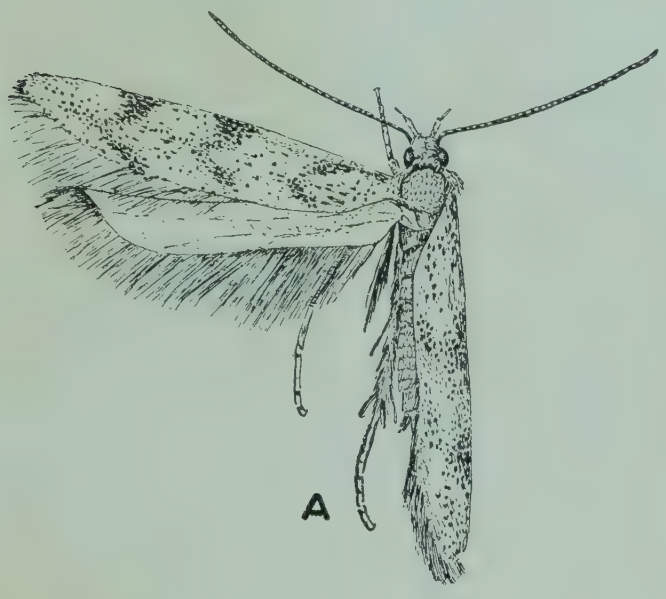
Recurvaria milleri:

A.—Imago. (Drawn by Miss E. Armstrong.)

B.—Twig of lodgepole pine showing the 1919 needle growth: *a*, Eggs and their location between scales at base of needles. The needles which covered the eggs have been removed in order to expose them for photographing. Print slightly retouched. Approximately × 2.

C.—*a*, Lodgepole pine needle showing larval mine; *a1*, entrance hole of larva; *b*, larva exposed in needle mine; *c*, pupa in normal position in needle; *d*, parasitized larva in normal position in needle. Approximately × 2.

D.—Terminal twig of lodgepole pine showing partial defoliation The 1919 needle growth is at tip of twig and shows full complement of needles, as the photograph was made prior to the 1919 attack by the moths. The almost denuded section of the twig immediately below supported the 1918 needle growth, which was mined by the larvæ and has since fallen. The 1917 growth appears just below and shows as nearly a complete set of needles. Still farther down the twig the 1916 growth shows defoliation comparable to the 1918 growth section. Note difference in degree of defoliation every alternate year, the heaviest occurring the year preceding flight. This is accounted for by the fact that the larva mines two needles of this year's growth as compared to one needle of the growth produced in the year of flight, which receives the initial attack by the young larva. The same conditions are shown on the twig branch to the right.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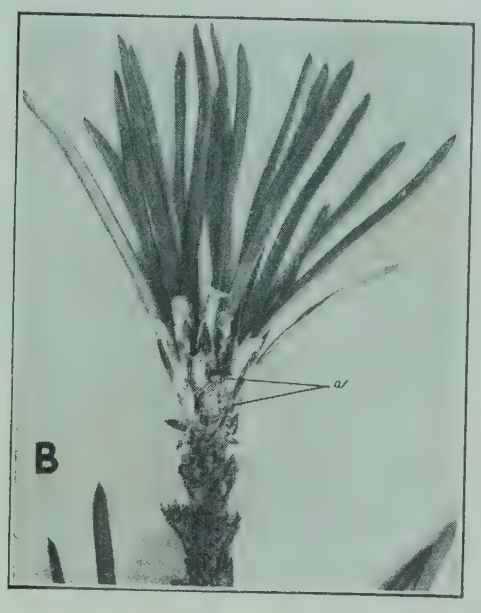
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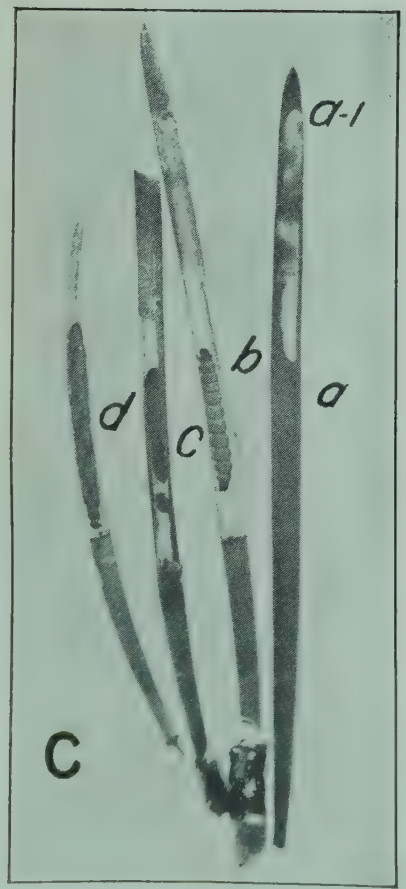
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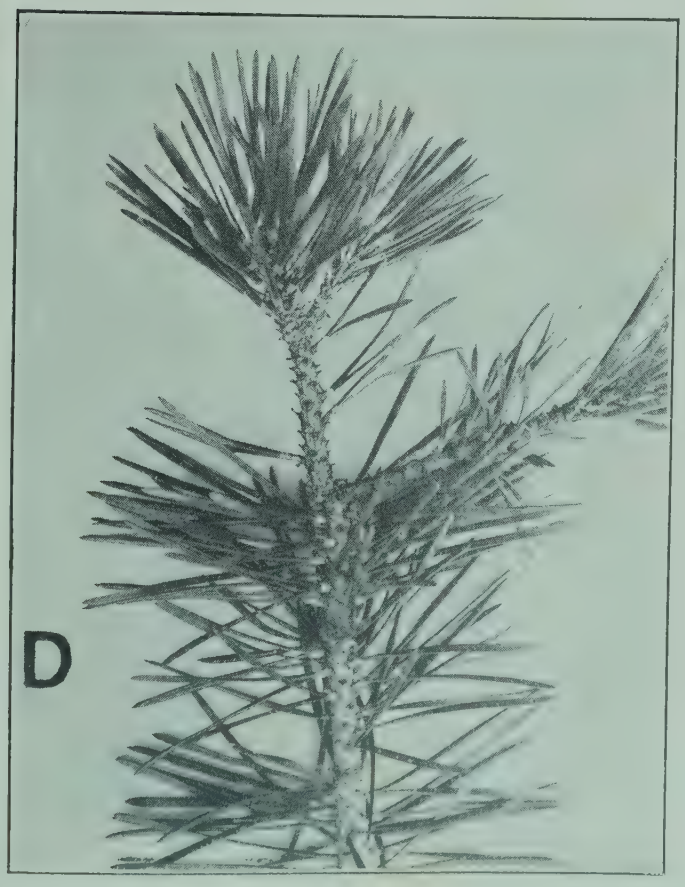
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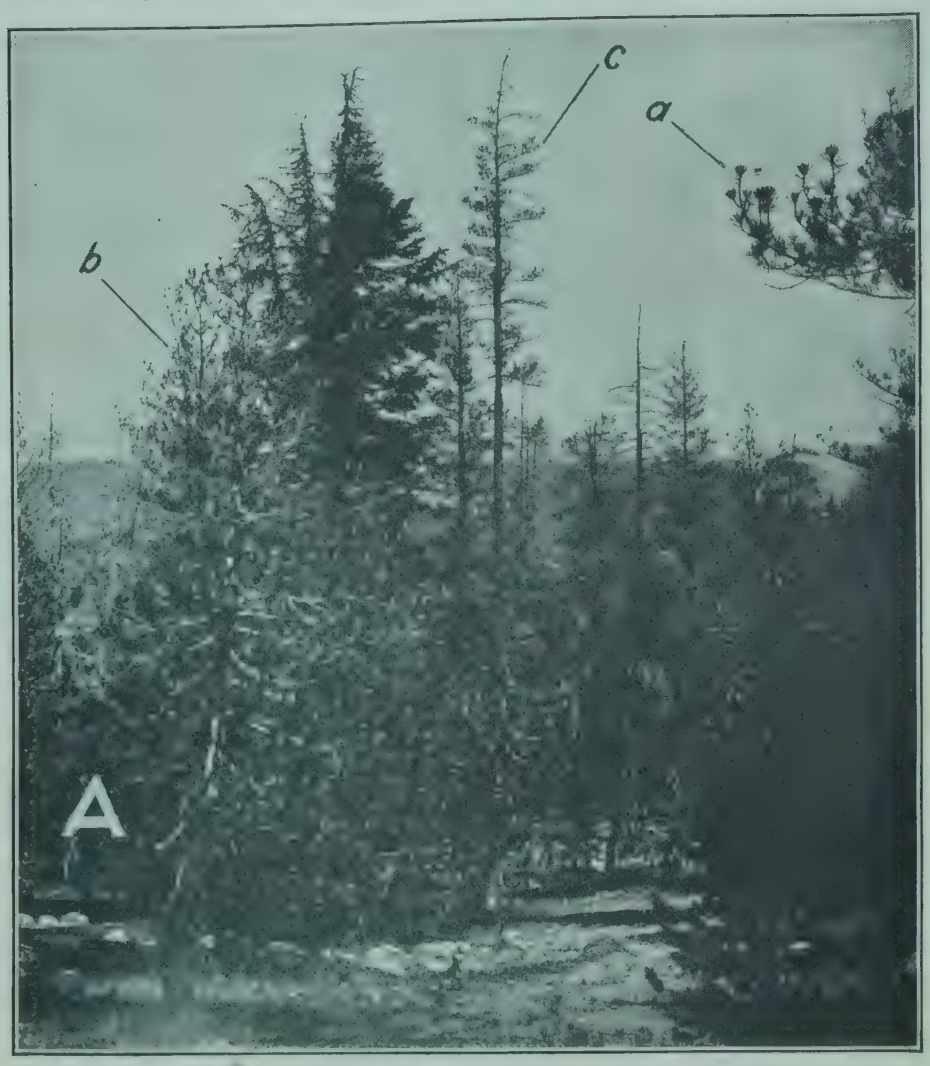
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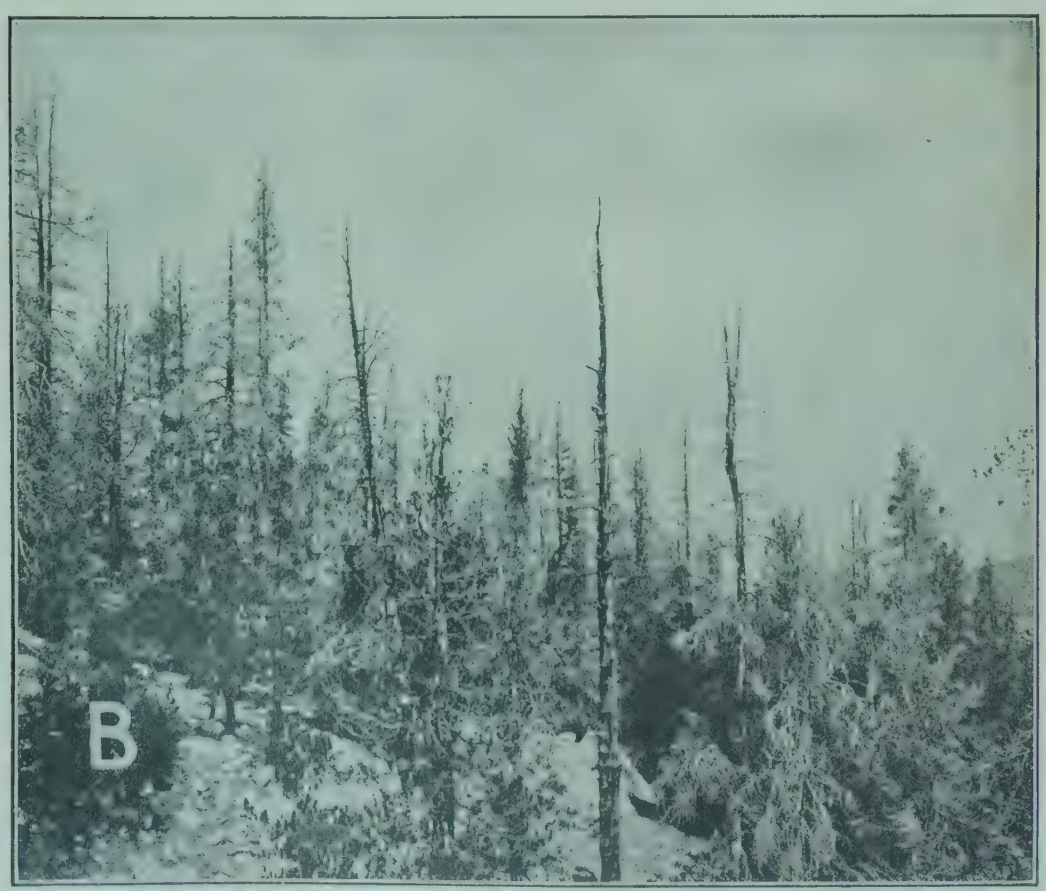
B

PLATE 30

Recurvaria milleri:

A.—Lodgepole pine stand showing damage. This stand has suffered for years by repeated defoliations. The defoliated condition of the lodgepole pine is plainly indicated by comparison with the mountain hemlock (the dark-colored tree showing full complement of needles). The three stages of defoliation are well illustrated in this photograph: *a*, First stage, indicated by partially defoliated twigs on limbs in upper right foreground. Only the 1919 growth on the tips of the twigs shows as full a complement of needles. The annual growth prior to 1919 has suffered to a greater or less degree by defoliation. This stage represents the initial stage of damage. *b*, Second or intermediate stage, shown by appearance of tree in left foreground. This tree has suffered severely by defoliation, many twigs being dead and the foliage almost entirely gone. The 1919 needle growth is stunted, indicating weakened vitality. *c*, Third and last stage, indicated by tall tree in center, just to right of hemlocks. Note the dead crown and limbs and almost entire absence of foliage.

B.—Lodgepole pine stand which has suffered severely by defoliation. Every tree has been repeatedly attacked for years. Note the dead crowns and limbs and scarcity of foliage. The top of the small tree in the foreground shows heavy damage to young, vigorous trees, as well as to the mature trees in the background.

A BACTERIAL BUDROT OF CANNAS

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INTRODUCTION

The disease described in this paper was first observed by the writer in July, 1918, when the unsightly appearance of hybrid canna plants (*Canna indica* L.) in the public grounds in Washington (Pl. 31) drew attention to it. The most noticeable lesions at that time were large, irregular brown spots which caused distortions of the leaves. Close inspection showed that many plants were attacked to a greater or less degree; leaves of all ages were involved, and often young shoots were killed by the destruction of the bud. A microscopic examination of sections of diseased areas disclosed the presence of swarms of bacteria in the tissues; and, as no mention of the disease was found in literature, a definite investigation was undertaken.

APPEARANCE OF DISEASED PLANTS

This disease is essentially one of young tissues and moist conditions. This is evident from the virulence of the attack on buds as well as from the fact that infections make little headway on mature leaves.

The spots on the leaves vary in size from minute stomatal infections to ragged, brown, irregular areas extending several inches along the blade, usually between midrib and margin (Pl. 32, 33). Small stomatal infections are found in great numbers near the margins of large spots or on leaves with no large lesions, and on mature leaves they do not develop further. On young leaves they enlarge into spots which tend to run between the parallel veins, giving to the leaf a striped effect. This effect is continued in the uneven margins of large spots formed by the coalescing of small ones, where often a diseased strip between two veins runs an inch or more beyond the main diseased area into otherwise healthy tissue. Young infections and the advancing edges of larger spots are first water-soaked, then yellow, later becoming brown. In the early morning water-soaked streaks extend far beyond the yellow tissue. Old spots are dry, thin, and grey-brown, and by shrinking cause distortions of the leaves (Pl. 32, 33). They show a dark, almost black, mottling or checkering, which is more plainly seen by transmitted than by reflected light, the

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different colored areas being rectángular rather than round. This mottled appearance and the position of the spots between midrib and margin clearly distinguish this disease from the common dying back of the older leaves, where the margin is first to succumb and the dead areas are a uniform red-brown.

Infection usually takes place while the leaves are still rolled in the bud. In case stomatal infections occur just before the leaf pushes up, the whole bud often looks very pale, almost white, and close examination shows it to be covered with minute white spots. Sometimes these do not spread further, and the leaf as it matures becomes green with a peppering of tiny spots, but more often as it unfolds it remains pale and stunted. At other times the infection has progressed so far that by the time the folded leaf emerges it is wholly or in part blackened, sometimes in spiral bands (Pl. 35, B). In such cases the younger leaves usually become infected by direct contact, or the disease runs down the petiole and kills the young stalk and bud. A non-fatal bud infection is shown in Plate 35, A, where blackening has occurred but not to such an extent as to kill the shoot.

From leaf-blade infections (Pl. 33; 36, C) the bacteria invade the petiole, not by way of the vascular system but through the parenchyma, chiefly through the channels of very loose tissue which occupy a large portion of the interior of the petiole. In the tightly rolled buds infection appears to pass directly from one leaf to another, so that when a young shoot is cut across near ground level, several petioles may be found to be diseased. The buds do not become soft-rotted but usually stand up black and dry or are bent over or broken off. Eventually the center rots out, leaving the hollow stalk standing with one or two mature leaves. This when cut across near the base is found to contain a watery rot. The rootstocks have never been found to be diseased. In the autumn of 1919 when the plants in the grounds were lifted for winter storage all the plants of one large bed that had shown heavy infection were thoroughly examined. All stalks were cut across a few inches from the ground, and in a large proportion of the clumps one to four stalks were found with interiors rotted out, to or below ground level, the lower part of the cavity being filled with fluid. In no case, however, was the rot found extending into the rootstock, the tissues of which do not seem to favor the growth of the organism. In many stalks showing a characteristic top—that is, standing erect with older leaves intact but with a hollow blackened center, the decay had not reached the lower part, so that a cross section 1 foot from ground level showed only sound tissue.

Often when the shoot has escaped early and complete destruction, the flower clusters are ruined either by the infection of the young flower buds or by the decay of the stem. In the former instance the stem and pedicels develop but the buds blacken and die while still rudimentary (Pl. 34, A, C). In the latter case the stalk bends or breaks in the infected

region (Pl. 34, B). Infection often remains on one side of the stalk, which blackens and, if infected very young, fails to elongate like the healthy side and so cracks across at frequent intervals, the cracks becoming gummy with the exuded sap (Pl. 36, C). Sometimes the rot extends along the stalk to its tip, blackening pedicels and well-formed buds.

SUSCEPTIBLE VARIETIES

During the summer of 1918 most of the varieties observed were healthy or showed only a trace of this disease. The badly affected sorts were Princeton, Gayety, City of Portland, and Charles Lutz. The disease was most virulent in the early summer, many plants recovering during August.

In 1919 the outbreak was much more virulent than in the preceding year, but the susceptible varieties were different. This time the Yellow King Humbert, a sport from the Red King Humbert, and Carmine Beauty were most injured. Many of the varieties planted in 1918 were not set out in 1919, so no comparison could be made. It was learned from one of the gardeners that some varieties had been dropped in the past because of this budrot, among them Fire Bird and Mrs. Alfred Conard. Another gardener ascribed all the trouble to overwatering and crowding in the hothouse before setting-out time, conditions undoubtedly very favorable to the activities of the causal organism.

DAMAGE DONE

In 1919 the disease was observed earlier than in the previous year—that is, in the latter part of May soon after the plants were set out. At this time there were only scattering infections, a few large leaf spots, and several infected and dead shoots. Later (June 19) several beds showed from 10 per cent to 80 per cent of infected plants; of these many had two or three of the four shoots involved, and eight plants in one bed had bent blossom stalks.

During July many plants outgrew the disease by sending out new, vigorous shoots; but in August, although to the casual observer no traces of disease were present, a great many unsightly leaves and some sickly young shoots might be found, and often a blossom stalk pushed up through a ragged brown sheath.

In May, 1920, potted plants in the hothouse ready for setting out were examined, and the following varieties were found severely infected—that is, with a scattering of dead or diseased buds: Yellow King Humbert, Gayety, Golden Eagle, Dazzler, Favorite, and Wallace.

Other varieties on the same bed and subject to the same conditions were entirely free from signs of the disease. These were Meteor, Olympic, Rosea Gigantea, Fenal, President, Princeton, and City of Portland.

No connection could be traced between infected beds of one year and the serious attack of the following year. The beds most heavily infected in 1918 were in some cases almost disease-free in 1919, others were badly

infected, while some beds where no disease occurred in 1918 showed the highest percentage of infection in 1919. These observations, however, are complicated by the fact that different varieties, the susceptibility of which is not known, were planted the second year. From present knowledge it seems that the disease must be carried over on the rootstocks, especially since the trouble begins to develop before the plants are taken from the hothouse.

Overwatering of the foliage appears to be a large factor in the development of the disease, since rootstocks which were taken from heavily infected clumps and kept under favorable conditions during the winter and carefully watered when potted in early spring gave not a single case during the entire summer, while rootstocks from the same source without special care showed a large percentage of infection before setting-out time.

GEOGRAPHICAL DISTRIBUTION

The disease has thus far been observed only in the District of Columbia and in Illinois. Typically infected plants were found at Urbana, Ill., in the summer of 1920.

ISOLATIONS

When sections were made of diseased tissues, motile bacteria were invariably found in great numbers swarming out on the slide. Plates were poured on peptone-beef agar from leaf spots, pedicels, and from petioles near the ground level. In every case practically pure cultures of a white bacterial organism were obtained. Judged from plate colonies, the organism was the same in every case, and comparative cultural studies of several isolations have corroborated this judgment.

INOCULATIONS

Inoculations were made in 1918 on potted cannas in the hothouse. These plants were not in good condition but were the only ones available at the time. Young leaves just unfolded were inoculated by placing drops from a young agar slant culture on the surface and making delicate pricks in the blade through these. Drops were also poured into the tips of tightly rolled leaves without wounding. Subcultures of isolations from leaf blades and from petioles were used. Some plants were kept moist for 36 hours by spraying in cages with sterile water; others were left in the open hothouse.

In most cases no infections appeared. One inoculated leaf showed on the fourth day several water-soaked streaks 1 to 10 mm. long running from needle pricks. These turned yellow then brown, but did not spread further. Plates were poured from the edge of the longest streak, and colonies were obtained which appeared to be right. Transfers from these conformed to the original isolation in subsequent cultural tests.

Attempts were made to infect the slow-growing cannas with the reisolation recorded above, but all of these failed. Further inoculation

work was therefore postponed until the following spring, when more favorable conditions would obtain.

When the disease appeared in 1919, isolations were made from active young infections on leaves and petioles; and single colony subcultures from these were used for inoculating young vigorous cannas, obtained from a new source, in large pots in the hothouse. Suspensions from young agar cultures were sprayed into the youngest rolled leaf of some plants without wounding. In other cases the stalk below the lowest leaf blade was smeared with bacteria, and pricks were made through this to the young leaves within. Part of the inoculated plants of each lot were kept in cages and sprayed with sterile water for 36 hours; others were left in the open house. Controls in other cages were sprayed with sterile water.

Good prompt infections were obtained by both spray and prick inoculations on the plants that were kept in cages, and only fair infections on those in the open house that were pricked. Controls remained healthy. Infection was apparent on the sprayed plants only when the young, susceptible leaves which were tightly rolled at the time of inoculation emerged or unrolled, usually after six or seven days. On the oldest rolled leaves those spots which at this time showed as small stomatal infections did not progress further. On younger leaves the initial stomatal stage was past, the spots extending from vein to vein and beginning to lengthen into streaks (Pl. 36, A). On pricked plants kept in cages, infection was more rapid and destructive, as is shown on Plate 36, B, C. Here infection showed on the fourth day, running downward from pricks seen in the photograph near the tips of the leaves (X, X). These leaves were tightly rolled when pricked through the enveloping folds of older leaves. In the leaf shown on Plate 36, C, the streak from the pricks on the midrib was 3 cm. long on the fourth day. One day later it was 10 cm. long, and by the eleventh day it had reached almost to the base of the next older leaf, widening downward where inclosed by the sheathing petioles and killing the shoot completely. After the plants were once infected, secondary infections took place in some cases on young shoots which were in the same pots with inoculated shoots but which were too young at the time of inoculation to have been directly infected—that is, were without any leaf which had begun to unfold. Younger unfolding leaves on sprayed shoots also showed infection as they emerged some weeks later.

From several of these infections reisolations were made, and inoculations with single colony transfers thus obtained gave typical infections on cannas when inoculated by spraying and by needle pricks. These isolations and reisolations were used for cultural work in comparison with cultures of the previous year, with which they were found to agree.

THE ORGANISM

DESCRIPTION

The causal organism is a short rod with rounded ends, single, in pairs or chains, 1 to 2 μ long by 0.5 to 0.7 μ broad, when stained from 24-hour agar cultures. It is motile by means of one to three bipolar flagella (Pl. 38, B). It does not form spores, is Gram-negative, is not acid-fast and stains readily with the ordinary anilin stains. Capsules were stained from 10-day agar cultures with Ribbert's stain. Rods with swollen ends occur in old milk cultures.

CULTURAL CHARACTERS [1]

AGAR PLATES.—On +15 (Fuller's scale) peptone-beef agar at 20° to 25° C., colonies appear on the second day. By the fourth day the surface colonies are 2 mm. in diameter, thin, white, round with entire margin, wet shining, finely granular, semitransparent, with internal concentric markings by oblique light, especially in the thinner margins. As colonies enlarge (5 to 8 mm.) they are white, slightly convex, and may be either round or irregularly scalloped (Pl. 37, C). The scallops are formed by wedges of more transparent growth in which distinct radiating lines are seen by direct transmitted light (Pl. 38, D). By oblique transmitted light the wedges show both radiating lines and also internal concentric markings (Pl. 38, E). In consistency they are viscid, becoming more so with age. Buried colonies are lenticular, becoming round to irregular.

AGAR STABS.—In agar stabs the surface growth is flat, wet shining, moderate, at first round, later with an undulate margin, then covering the entire surface. Stab growth is moderate, granular, tapering downward, ending at one-half the depth of the agar. In old cultures crystals form from the surface downward in ragged spears 1 to 2 cm. long. There is no discoloration of the agar.

AGAR STREAKS.—Two-day-old streaks from bouillon are filiform, 2 mm. wide, tapering upward, white, wet shining, with thin margins and granular center. Later (6 days old) growth is 4 to 5 mm. wide with finely scalloped edges and radiating lines by transmitted light, running from the granular center into the translucent margins. The V is half filled with white precipitate. The growth is very viscid.

GELATIN PLATES.—On gelatin plates kept at 15° C. colonies appear on the fourth day. At 20° they are visible on the second day. Growth is very slow and without liquefaction at 15°. Colonies are thin, round, later becoming flower-like—that is, with a crater-like center and wider scalloped margin (Pl. 38, C). At 21° to 24° very slow liquefaction occurs, beginning about the tenth day, especially on thickly sown plates. On thinly sown plates colonies usually lie in a shallow, dry saucer.

[1] Kahlbaum's agar and Difco peptone were used throughout.

GELATIN STABS.—There is slight granular growth along the entire line of puncture, best at the top where it is sometimes villous or papillate, the length of the villi or papillae decreasing downward. Surface growth when 6 weeks old at 15° to 18° C. is thin, white, transparent, slightly rugose, with an undulate margin. No liquefaction occurs within two months at this temperature, but at 21° to 25° a saucer of liquefaction 1 cm. deep may be formed within three weeks. Usually clusters of crystals form at the bottom of the liquefied part.

WHEY AGAR [1] PLATES.—Colonies on whey agar plates are white, round, convex, opalescent, with internal concentric markings by oblique light. On thickly sown plates by the third day, and on thinly sown plates by the fourth or fifth day, each colony is surrounded by a clear area 1 to 2 mm. wide, beyond which a white halo extends outward (Pl. 37, B). On thickly sown plates this involves the whole surface. As the colony grows it fills the clear space, even growing out into the white halo. The halo is composed of an alkaline precipitate, which is readily dissolved by acids.

WHEY AGAR SLANTS.—Streaked from beef bouillon, growth is moderate, white, filiform, 2 to 4 mm. wide with undulate margins. The whole surface of the agar becomes white-clouded except for a clear area 1 to 4 mm. wide closely surrounding the streak of growth (Pl. 37, D). This halo is dissolved by acids.

POTATO CYLINDERS.—Growth on steamed potato is scanty, spreading, dirty white, wet shining, transient, becoming pale brown. The potato is grayed. Diastasic action is feeble.

BEEF BROTH.—Peptonized beef broth (+15) clouds weakly within 24 hours at room temperatures (21° to 25° C.); often within this time it forms a heavier flocculent surface layer, which falls on the slightest agitation. In undisturbed cultures the clouding is often banded, the heavier bands at the top. A heavy, viscid pellicle forms, which often falls slowly, center first, the edges remaining attached to the walls, so that a hollow inverted cone is formed (Pl. 37, A), which lengthens slowly to reach from pellicle to precipitate, and may persist for weeks. This occurs in both alkaline and acid bouillons. The abundant viscid precipitate is granular, semitransparent, and does not form a compact mass. It rises in a tenaciously viscid swirl on shaking. Clouding becomes heavy and is persistent.

MILK.—Milk begins to clear on the fifth to the tenth day, and clearing is complete within four weeks. No coagulation takes place. The milk becomes golden brown on long standing, sometimes with a jelly-like consistency.

[1] Formula for whey agar: To 3 pints of milk heated to boiling add a 20 per cent solution of hydrochloric acid sufficient to coagulate the milk. Avoid excess of acid. Filter through cheesecloth. Add *N*/4.5 sodium hydroxid until the whey titrates +7. To 500 cc. of whey add 150 cc. of water, 1.5 gm. of Nelson's photographic gelatin No. I, 7.5 gm. of peptone, 7.5 gm. of agar flour, and 7.5 gm. of saccharose. Dissolve by steaming 20 minutes, clarify with white of egg, tube, and autoclave 15 minutes at 110° C.

LITMUS MILK.—Litmus milk blues rapidly and uniformly from the top downward, beginning on the second day. There is no coagulation, but a gradual uniform clearing. Reduction begins promptly, the color passing from blue to pale purplish gray (Ridgway,[1] *Pl. LIII*), drab gray (Ridgway, *Pl. XLVI*), light drab (Ridgway, *Pl. XLVI*), and tawny olive (Ridgway, *Pl. XXIX*). Later the blue color returns.

METHYLENE BLUE MILK.—Milk to which methylene blue was added to make it robin's egg blue shows reduction in color on the second day. In 10 days reduction is complete.

COHN'S SOLUTION.—Usually no clouding occurs in Cohn's solution. Occasionally very weak clouding takes place and a faint rim is formed. After six weeks in such cultures the fluid is clear with 3 to 6 mm. width of white precipitate which breaks up on shaking.

USCHINSKY'S SOLUTION.—In Uschinsky's solution clouding is heavy with a viscid pellicle falling like that in beef broth, and a heavy viscid precipitate.

FERMENTATION TUBES.—In fermentation tubes containing 1 per cent peptone plus 1 per cent saccharose, dextrose, lactose, maltose, glycerin, or mannit there is good clouding in the open end, but none in the closed end, and no gas. All give a slightly alkaline reaction to neutral litmus paper at the end of two weeks.

LITMUS SUGAR AGAR.—Litmus agar containing 1 per cent peptone water plus 1 per cent saccharose, dextrose, lactose, maltose, galactose, or glycerin is not reddened. Moderately good growth occurs on all.

BLOOD SERUM.—Growth on blood serum is moderate, with no liquefaction and no discoloration of the medium.

CONGO RED AGAR.—On congo red agar little or no growth occurs, but in a few cases it was sufficient to show that the red color is taken up by the organism.

OPTIMUM REACTION FOR GROWTH IN BOUILLON.—The organism grows best in +10 to +15 peptone-beef bouillon. It grows fairly well in +20 to +25 but not at all in +30. Growth is good in neutral bouillon and in −5 to −10, is weak in −15, very weak in −20, and no growth occurs in −25.

TOLERATION OF ACIDS.—Neutral peptone-beef bouillon was used, to which was added 0.1 per cent, 0.2 per cent, and 0.3 per cent of malic, tartaric, and citric acid, respectively. These titrated as follows:

Malic	0.1 per cent	+18	Tartaric	0.1 per cent	+23	Citric	0.1 per cent	+24
	.2 per cent	+32		.2 per cent	+37		.2 per cent	+40
	.3 per cent	+47		.3 per cent	+53		.3 per cent	+53

The organism grew readily in 0.1 per cent of all three acids, best in the malic, and weakest in the citric. No growth took place in 0.2 per cent or in 0.3 per cent of any of them. Other tests were made with

[1] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 pl. (col.). Washington, D. C. 1912.

the following results: Growth in +32 malic acid, +37 citric acid, and +35 tartaric acid; no growth in +33 malic acid, +40 citric acid, and +37 tartaric acid.

TOLERATION OF SODIUM CHLORID.—Tests were made in +15 peptone bouillon to which were added 1, 2, 3, 4, and 5 per cent sodium chlorid. Prompt clouding, becoming heavy, appeared in 1 per cent, and fairly prompt moderate clouding appeared in the 2 per cent. The growth in 3 per cent was delayed and took the form of cobwebby, viscid, persistent, streamers without clouding. These streamers were not made up of chains but of single or paired organisms. No growth occurred in the presence of 4 or 5 per cent sodium chlorid.

OTHER CULTURAL FEATURES.—Growth is not retarded in bouillon over chloroform. Nitrates are strongly and promptly reduced. No indol is formed (10 days to 2 months). Hydrogen sulphid is produced in peptone-beef bouillon. Ammonia production is strong. The odor of most cultures is rather disagreeable.

TEMPERATURE RELATIONS.—The optimum temperature for growth is about 35° C. No growth takes place in peptone-beef bouillon (+15) below 5° nor above 40°. In one test there was very weak growth at 40°. The thermal death point is 52°.

EFFECT OF FREEZING.—When transfers from young +15 peptone bouillon cultures are frozen solid and kept frozen for 15 minutes, then thawed and plates poured with measured loops just as before freezing, the colony counts show that from 50 to 90 per cent are killed.

EFFECT OF DESICCATION.—The organism is very sensitive to drying. Drops of 1- to 6-day-old bouillon cultures were dried on cover glasses in sterile Petri dishes in the dark. These covers transferred to bouillon after 2 days' drying gave prompt clouding; after 3 days less than half gave growth, and after 5 days no growth was obtained.

EFFECT OF SUNLIGHT.—The organism is very sensitive to sunlight. Agar poured plates, one-half covered with black paper, were exposed to bright sunlight bottom side up on ice in November at 11.30 a. m. When counted 5 days later, colonies were numerous on the covered side. On the exposed side there was noticeable reduction after 1 minute's exposure; 75 per cent were killed after 2 minutes, 95 per cent after 3 minutes, and all were killed after 4½ minutes' exposure.

VITALITY ON CULTURE MEDIA.—The most long-continued growth is made in milk, peptone-beef bouillon, and peptone-beef agar. At room temperatures the organism will live in these media for 6 or 7 months, or until the medium is almost completely evaporated. Cultures in these media kept in the ice box for 1 year give prompt growth when transferred.

GROUP NUMBER

According to the chart of the Society of American Bacteriologists [1] the group number of this organism is 211.3333023.

TECHNICAL DESCRIPTION

Bacterium cannae, n. sp.

A short rod with rounded ends; chains; flagella 1 to 3, bi-polar; capsules; no pseudo-zoogloeae; aerobic; nonchromogenic; liquefies gelatin very slowly; diastasic action weak; reduces nitrates; does not produce acid or gas from sugars; clears milk; blues, then reduces litmus milk without coagulation; does not produce indol; produces hydrogen sulphid and ammonia; grows in Fermi's and Uschinsky's solutions and very feebly or not at all in Cohn's solution; optimum temperature 35° C., maximum 40°, minimum 5°; thermal death point 52°; vitality at room temperatures on media six months; Gram-negative, not acid-fast; sensitive to drying; moderately tolerant of acids and alkalis; sensitive to freezing and to sunlight. The cause of a meristematic disease cultivated in cannas.

SUMMARY

The budrot of cannas is a hitherto undescribed bacterial disease caused by *Bacterium cannae*, n. sp. The disease is primarily one of young tissues and moist conditions.

Infection takes place through the stomata and spreads through the intercellular spaces of the parenchyma of leaf blade, petiole, and stalk.

It is most destructive early in the season, that is on young plants. It begins in the hothouse and continues in the open beds. It destroys the buds, forms large unsightly spots on the leaves, and ruins the blossom clusters by blighting the flower buds or by decaying the stalk.

The method of overwintering whether in the soil or on the rootstocks, or both, is uncertain as yet. Although no means of control has been worked out, it is recommended as a preventive measure that rootstocks for planting be selected as far as possible from healthy stock only, that care be observed to avoid crowding and overwatering before setting out, that good ventilation be maintained in the houses, and that specially sensitive varieties be discarded.

[1] SOCIETY OF AMERICAN BACTERIOLOGISTS. DESCRIPTIVE CHART. Indorsed by the society for general use at the annual meeting Dec. 31, 1914. Prepared by the committee on revision of chart identification of bacterial species.

PLATE 31 [1]

Young canna shoot (natural infection) in which the bud has been killed. *Bacterium cannae* was plated from the interior 1 inch from the base of the shoot. Root-stock healthy.

[1] All photographs in Plates 31 to 38 are by Mr. James F. Brewer.

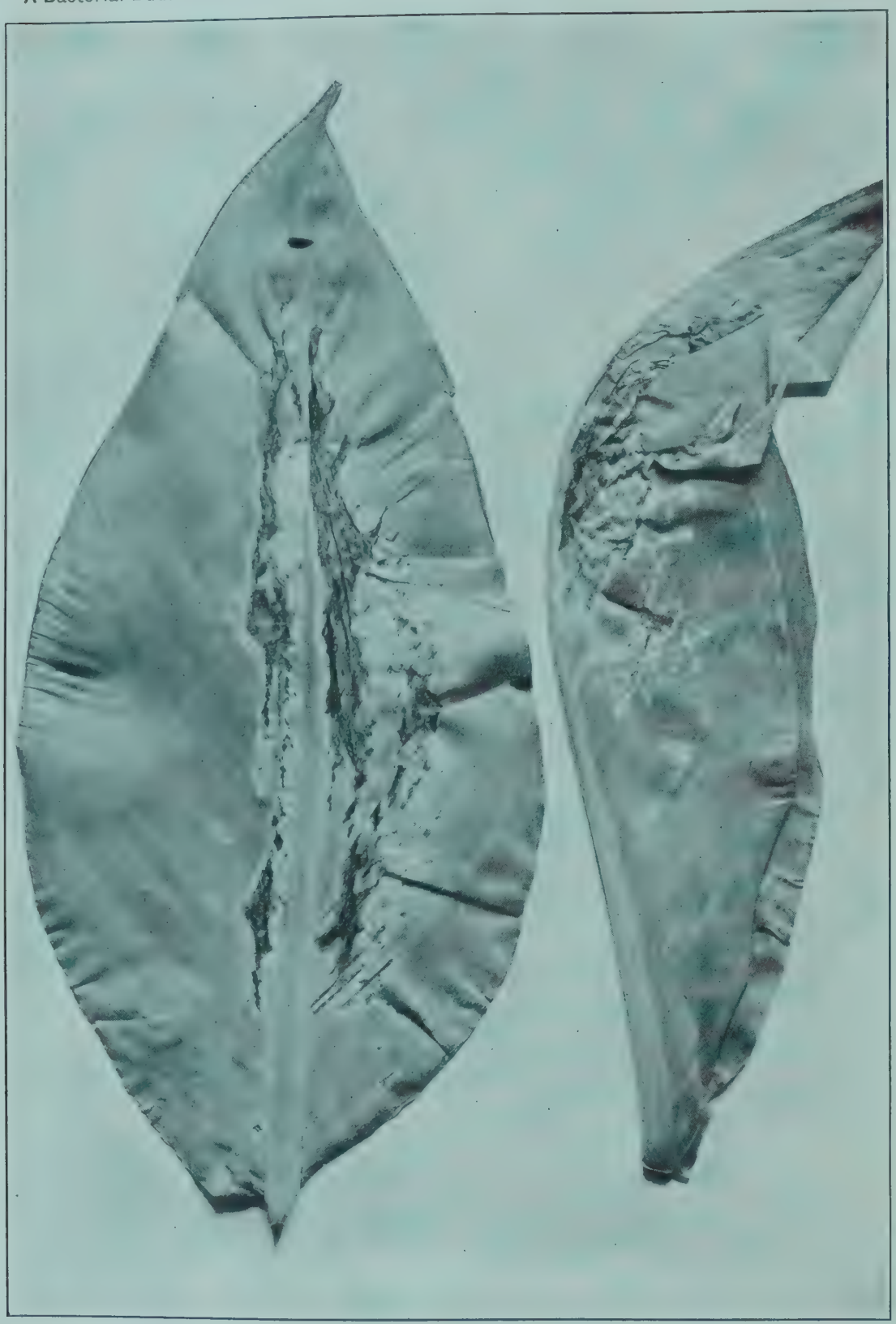


PLATE 32

Canna leaves (natural infection), showing character of spots and distortion of leaves. These were put under glass to straighten sufficiently for a photograph. Note lighter (yellowed) areas and minute stomatal infections in the vicinity of large spots. Much reduced.

PLATE 33

Canna leaves (natural infection), showing disease running down the petioles from large leaf spots. Compare Plate 36, A, where the infection is just reaching the midrib.

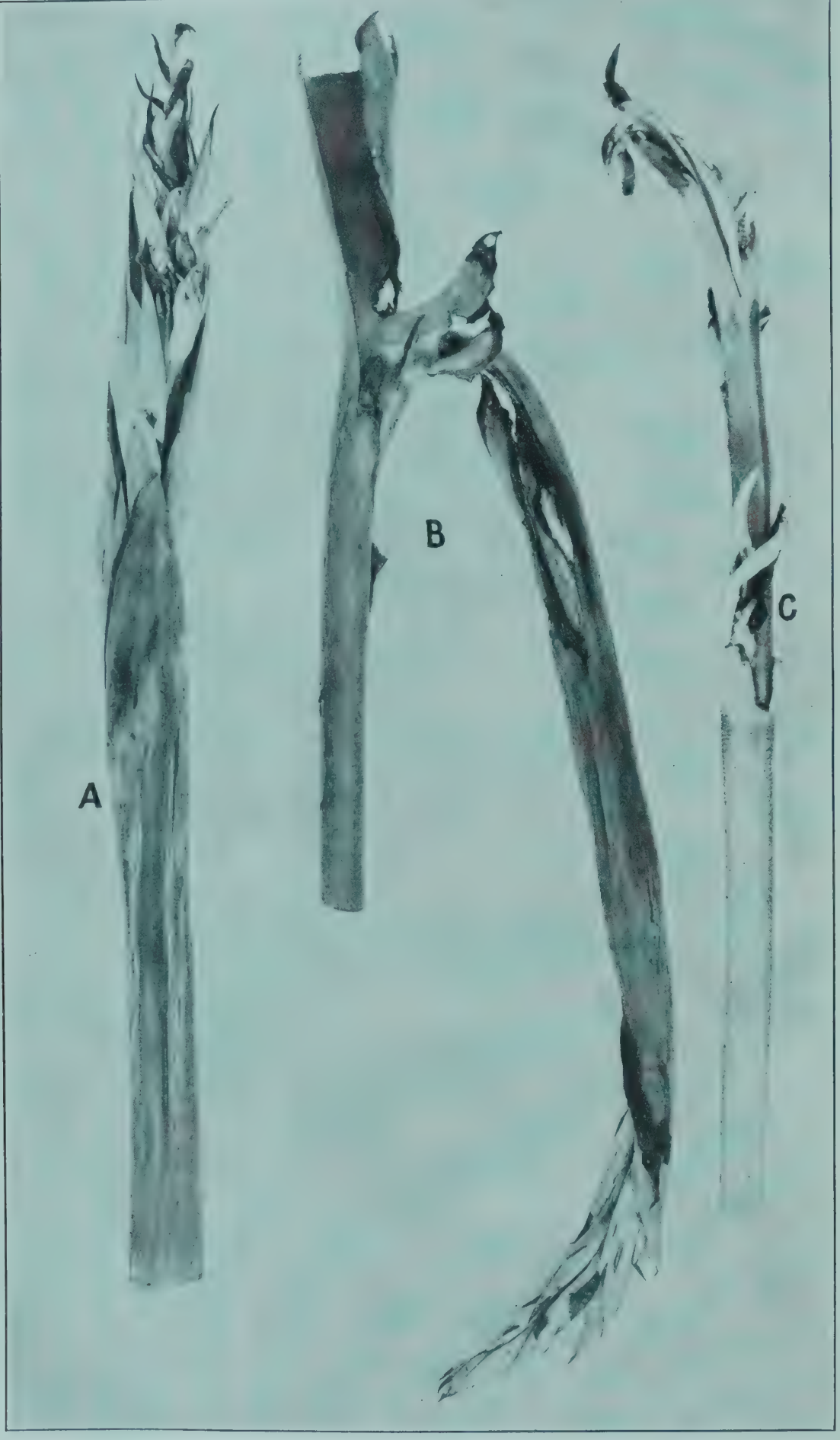

A
B
C

PLATE 34

Blossom clusters (natural infection).

A, C.—Infection on the blossom buds (blackened) of uninjured stalks.

B.—Stalk decayed on one side and broken over while the buds are only slightly infected.

PLATE 35

Tightly rolled buds showing infection.
A.—Bud moderately infected, and next older leaf with infections at base and at tip.
B.—Badly infected bud. Entire blackened area diseased.
Natural infections.

A
B

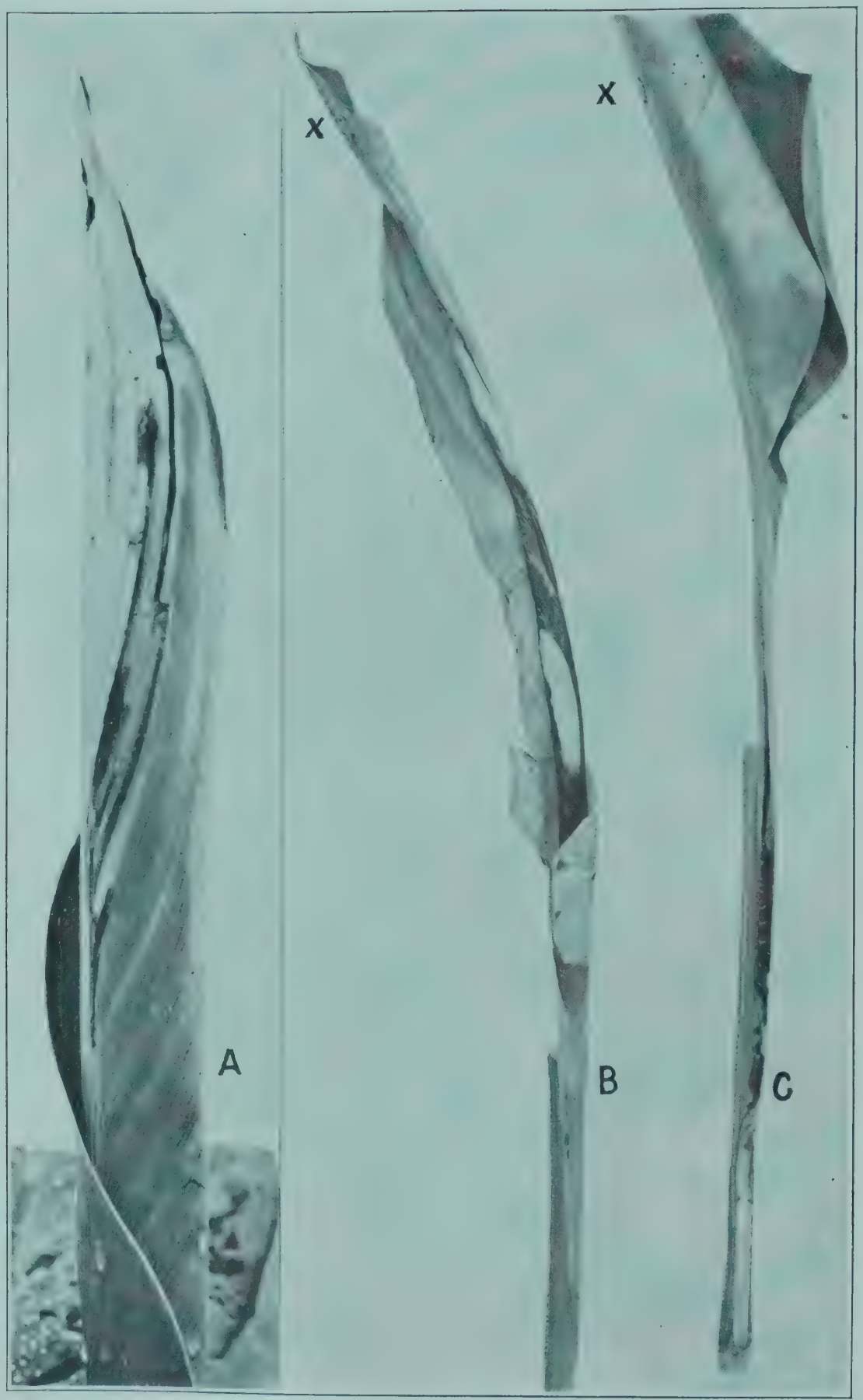

X
X
A
B
C

PLATE 36

Results of pure culture inoculations.

A.—Second infected leaf on a very young shoot inoculated by spraying, 18 days after inoculation. In 5 days infection ran from water-soaked spots near the tip into the midrib as shown in the figure.

B, C.—Needle prick inoculations 11 days old. Infection has run down from pricks in upper part of each. Blackened areas are diseased. Part of leaf cut away in both to show decay within. In C observe cracks in the blackened area caused by the stretching of the sound side.

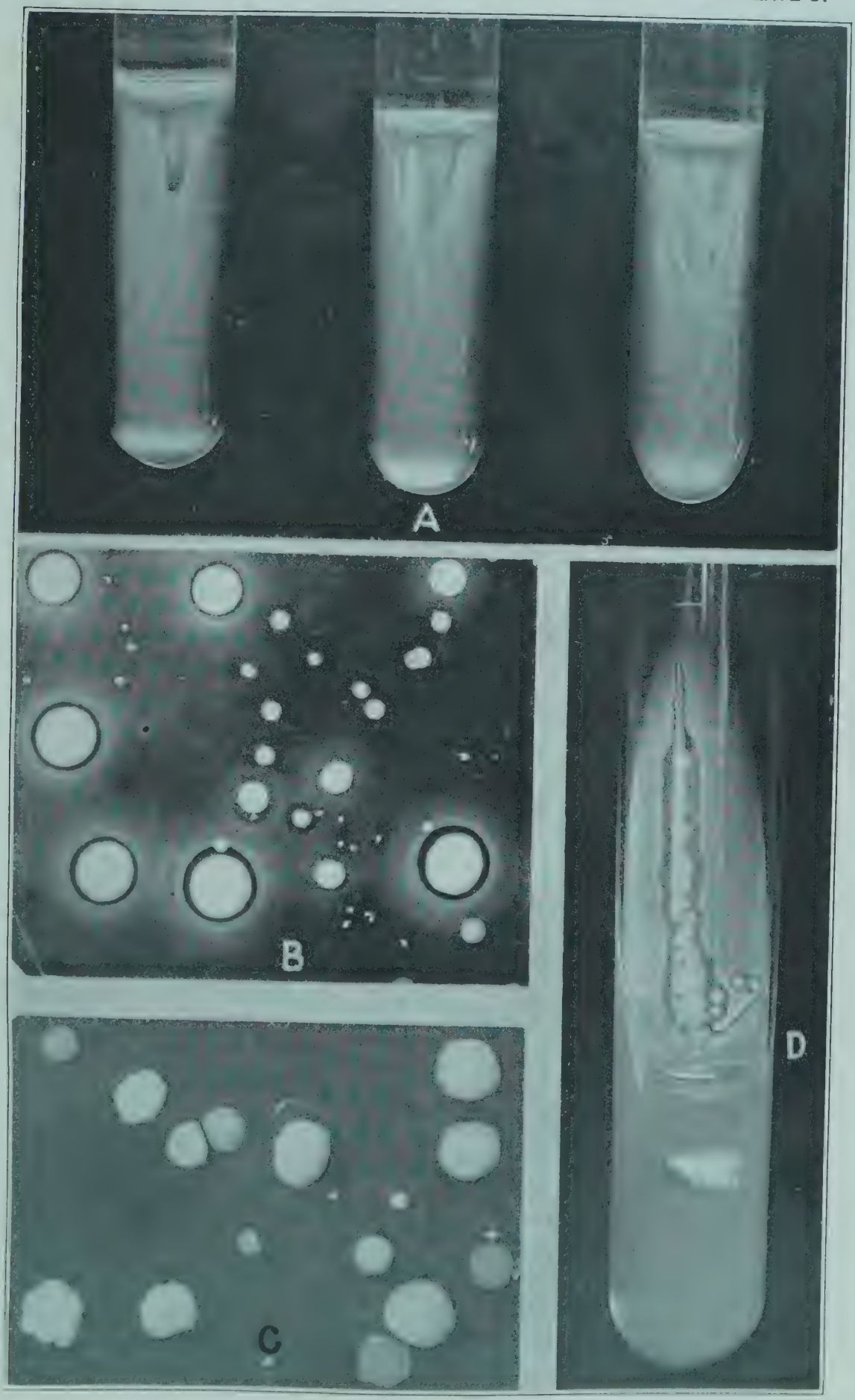
PLATE 37

A.—Cultures in + 10 beef bouillon, 8 days old, showing viscid, persistent, falling pellicle in different stages.

B.—Colonies on 8-day-old whey agar plates. × 2.

C.—Colonies on beef agar 11 days old, showing shape. × 2.

D.—Whey agar slant 3 days old, showing clear area and alkaline halo.


A
B
C
D

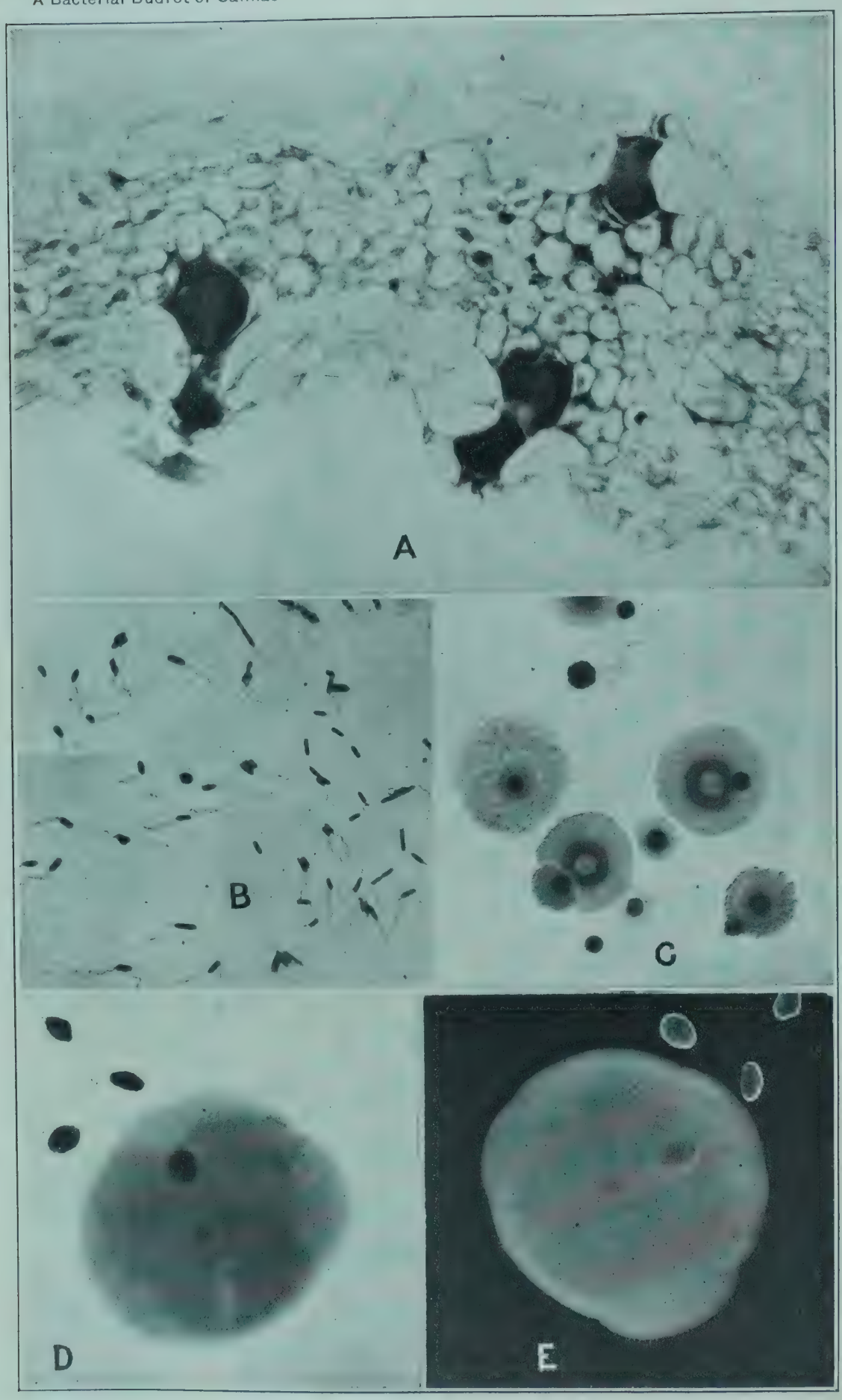

A
B
C
D
E

PLATE 38

A.—Section of young leaf with stomatal infections. Bacteria have penetrated the intercellular spaces to the center of the leaf from the upper infection at the right. The cloud below the lower left infection is composed of bacteria. Stained with Ziehl's carbol fuchsin.

B.—*Bacterium cannae*, showing flagella. × 850.

C.—Colonies on gelatin plate 7 days old at 15° to 18° C. × 10.

D.—Agar plate colony by direct transmitted light, showing radiating lines and 3 buried colonies. × 10.

E.—Same colony as in D but by oblique transmitted light, showing internal concentric markings and radiating lines. × 10.

A CHLOROSIS OF CONIFERS CORRECTED BY SPRAYING WITH FERROUS SULPHATE

By CLARENCE F. KORSTIAN, *Forest Examiner*, CARL HARTLEY, *Pathologist*, LYLE F. WATTS, *Forest Examiner*, and GLENN G. HAHN, *Scientific Assistant, Forest Service and Bureau of Plant Industry, United States Department of Agriculture* [1]

INTRODUCTION

In plants the term "chlorosis" is commonly applied to any abnormal condition whose most conspicuous symptom is a deficiency of green pigment. An exception to this general statement is perhaps the albinism of seedlings of oak, pine, and other plants which are from the first entirely lacking in chlorophyll, or, as sometimes happens in the conifers, have green cotyledons but no green in the leaves formed later. While such plants have always, so far as the writers' experience goes, died in the seedling stage, and the phenomenon must therefore be regarded as strictly pathological, the condition is not ordinarily spoken of as chlorosis. The inherited tendency on the part of healthy plants of horticultural varieties to grow leaves or parts of leaves lacking in chlorophyll is not usually considered pathological, and is better known as "variegation" than as chlorosis. True chlorosis may be due to a number of causes, such as low temperature, which hinders the formation of pigment, or lack of nitrates, which, according to Crocker (*2*),[2] at least in one of the algae, is associated with a rapid decomposition of chlorophyll. Plants in full sunlight are often less green than those less exposed, probably because of the rapid disintegration of the pigment in strong light. High temperatures very likely have the same effect (Blackman's "time factor"). Plants with deficient water supply are, on the other hand, liable to chlorosis caused by difficulty in pigment synthesis.

Much study has been given to the chlorosis of plants on calcareous soils, especially in connection with grapes in Europe. Roux (*18*) lists a large amount of literature on this subject. Recent papers by Mazé, Ruot, Lemoigne (*13*), and Gile (*6*) are well worth attention. The favorable effect of iron on plants affected with certain types of chlorosis was discovered before the middle of the last century, spraying a solution of an iron salt on chlorotic leaves having resulted in correcting the chlorotic appearance (*24*). Molisch (*14*) discusses many of the earlier experiments with iron. In a recent interesting paper, Johnson (*12*) states that spraying with iron salts is helpful for a chlorosis associated with extremely high

[1] The writers wish to acknowledge helpful suggestions from Dr. C. B. Lipman and Dr. Howard E. Pulling.

[2] Reference is made by number (italic) to "Literature cited," p. 170–171.

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manganese content of the soil. Dementjew (*4*) discusses the question of whether chloroses corrected by iron are really cases of iron hunger.

The literature on the chlorosis of conifers is relatively small. Sorauer (*23*) has reported chlorosis in *Thuja occidentalis* in Europe, and Schmuziger (*21*) and Dafert and Kornauth (*3*) have noted chlorosis in spruce, without attempting to connect it with causal factors. Schmuziger reports, as do other observers on angiosperms, that the chlorotic leaves contained plastids which became green when the leaves recovered. Neger (*16*) has described in more detail a chlorosis of spruce in a cold autumn in which the yellow leaves or parts of them were found to contain much more starch than the green leaves or their green bases with yellow tips. He rather vaguely connects both current low temperature and the drouth of the preceding winter with the various phenomena observed.

Contejean (*1*) lists Scotch pine (*Pinus sylvestris*) as somewhat calcifuge, and makes the general statement that excess lime accompanied by lack of iron, or "encore plus" lack of potassium, results in chlorosis of calcifuge plants. He, however, makes no specific mention of chlorosis in any conifer. Fliche and Grandeau (*5*) attribute the calcifuge tendencies of *P. sylvestris* to the physical rather than the chemical qualities of lime soils. They find Austrian pine (*P. austriaca*), *P. halepensis*, and *Abies pectinata* doing well on strongly calcareous soils, but they find *P. pinaster* making a poor growth in plantations on calcareous soil in all cases observed and entirely refusing to grow in some cases. Deficiency in starch and chlorophyll are noted for this pine on the lime soils, and also to a very slight extent for the Austrian pine on soils with extremely high calcium-carbonate content. The chloroplasts of the chlorotic plants are said to be small. The poor condition is attributed to potash hunger, and no mention is made of iron hunger as a possible cause. Ash analyses showed the following conditions:

On good soil,

Pinus pinaster, potash 16 per cent, iron oxid 3.8 per cent, lime 40 per cent.

On excessively calcareous soil,

Pinus pinaster, potash 5 per cent, iron oxid 2.1 per cent, lime 56 per cent.

Pinus austriaca, potash 14 per cent, iron oxid 3.3 per cent, lime 49 per cent.

Sachs (*20*) reports chlorosis in young trees of *Abies balsamea*, *A. apollonis*, and *A. bicolor* and says that entirely chlorotic new growth becomes green more or less promptly after considerable quantities of solid iron sulphate are placed in ditches in the soil near the roots. No controls are mentioned, but the promptness with which the younger trees are reported to have responded to the treatment supports his conclusion that the recovery was due to the iron added, despite the fact that fast-growing chlorotic shoots, according to his own statement,

usually improve in color toward the end of the season without any special treatment.

An interesting American report is that on chlorosis of *Sequoia sempervirens* by Peirce (*17*).

CHLOROSIS OF CONIFER NURSERY STOCK IN THE UNITED STATES

At several of the nurseries of the United States Forest Service in Nebraska and farther west, conifers are occasionally somewhat chlorotic The condition has become a matter of importance, however, only in the Morton Nursery, in northwestern Nebraska, and the Pocatello Nursery, in southern Idaho. Chlorosis has also been noted in conifers at the Great Basin Experiment Station in central Utah, especially in lodgepole pine (*Pinus contorta*) seedlings and transplants grown two years in the seed bed and one year in the transplant bed. At the latter locality native aspen (*Populus tremuloides*) was also chlorotic in places.

ANALYSES OF SOIL AND WATER

At all the points at which chlorosis was found, analysis (by courtesy of the United States Bureau of Soils for the nursery soils, and of Dr. J. E. Greaves, of the Utah Agricultural Experiment Station, for the Great Basin Experiment Station soils, showed the presence of carbonates as indicated by carbon-dioxid evolution. Carbon dioxid was, however, reported from sites near the Great Basin Experiment Station on which no chlorosis had been observed in either aspen or conifers, and from a nursery at which chlorosis had never been serious. In some cases the amount reported from soils on which the trees were green was greater than from those where the trees were chlorotic. The acid-digestion analyses showed for all the soils on which chlorosis was observed a considerable amount of calcium, much greater than that ordinarily found in the humid region of the United States, and in every case greater than the average of the 570 soils of the arid region reported by Hilgard (*10, p. 377*). However, there is little apparent correlation between the amount of chlorosis and the amount of calcium reported. The Utah soil on which conifers were not chlorotic yielded over 17 per cent of lime (as CaO) and 12½ per cent of carbon dioxid. The Pocatello nursery soil on which chlorosis was serious yielded more than twice as much calcium (2.9 to 4.7 per cent CaO) as Hilgard's average for arid soils. It was not an excessively calcareous soil, however, as compared with some of the soils reported in connection with chlorosis in Europe and Porto Rico, with the chalk soils reported by Somerville (*22*) on which healthy Douglas fir was growing, or with the Utah soil just mentioned as supporting normally green conifers. The phosphorus (as P_2O_5) for the Pocatello soil was reported as approximately 0.7 per cent for all the samples, an unusually high figure. This at once suggests a possible relationship, in view of the slight

solubility of ferric phosphate. The other soils on which chlorosis occurred, however, did not give any such high phosphorus analysis. The fact that the solubility of ferric phosphate is sufficient to make it a good source of iron in water-culture experiments prevents any probability of a relation between the amount of phosphorous found by analysis and the availability of iron.

All the analyses indicated normal quantities of iron. The results are in agreement with the general experience that acid-digestion soil analyses yield little information of value from the plant physiological or pathological standpoint. Petrographic examination by the United States Bureau of Soils of the Pocatello soil and of the nursery soil which contained carbonates without chlorosis gave no clue to the difference between the plants on them. Acidity determinations by Dr. L. J. Gillespie, of the Bureau of Plant Industry, showed a P_H of 7.8 for the Pocatello soil and 8.4 for the nursery at which there were carbonates but no serious chlorosis. The main facts to be drawn from the examination of the soils of the different stations was that all the soils on which chlorosis occurred contained carbonates and that two of them were underlaid with limestone.

Analysis by the United States Bureau of Chemistry of the water supply showed 320 mgm. of bicarbonic acid (HCO_3) per liter of water at the Pocatello Nursery, and practically no other anions, while at the nursery at which there were soil carbonates but no chlorosis there were reported 180 mgm. of bicarbonic acid per liter, as against 450 mgm. of sulphate (SO_4) per liter. This is of some interest in connection with the difference in the amount of chlorosis at the two places, as the arid conditions made necessary the application of considerable amounts of water to the nursery beds during warm weather. The soil solutions during the periods of greatest growth must, therefore, have been influenced to a considerable extent by the character of this water. It was noted at the Pocatello Nursery that the chlorosis was more prevalent in beds which had been under nursery management for several years than in beds which had just been included in the nursery area and had therefore received less of the water.

KIND AND EXTENT OF INJURY

At the Pocatello Nursery there was so much chlorosis and the growth of affected stock was so unstisfactory that a detailed study of it was undertaken. The nursery is at an elevation of 5,200 feet, well below the lower limit of natural coniferous forest growth in this region. Precipitation for the period during which the nursery is usually free from snow (April to October, inclusive) averaged but 11.2 inches for the years 1909 to 1917, inclusive. The days are warm and the nights cool during the growing season, only 2½ months being entirely free from killing frost. The soil is a rather heavy black silt loam; composite samples from 8 to 10

points each show for three different parts of the nursery wilting coefficients of 11.7, 12.6, and 14.3 per cent, respectively.[1]

The species in which the chlorosis has been noted are western yellow pine (*Pinus ponderosa*), Jeffrey pine (*Pinus jeffreyi*), Corsican pine (*Pinus laricio corsicana*), and Douglas fir (*Pseudotsuga taxifolia*). Of the two most important species grown here, western yellow pine and Douglas fir, the former is the more susceptible, especially during its first year. During the second year, Douglas fir is also considerably affected.

The yellowing first becomes evident in the leaves of most recent growth, as reported by Sachs (*20*) for firs and broad-leaved plants. The entire foliage may be affected. In serious cases, the leaves are short, inclined to curl, and are less turgid than normal leaves (as a consequence of lack of sugars and therefore low osmotic pressure). The terminal bud either fails to develop or is dwarfed and usually abnormally light in color. The height and diameter of the stem, the length of the roots, and especially the ability to form fibrous lateral roots also appear to suffer in typical cases of chlorosis. The disease may occur in patches, or isolated yellow plants may occur. In severe cases death ensues, the parts first discolored being the first to die.

The greater part of the injury develops after height growth has mainly ceased for the season. A marked functional disturbance is indicated in the apparent inability of chlorotic plants to harden properly for the winter. Chlorotic first-, second-, and third-year seedlings of both Douglas fir and western yellow pine, though not growing with the vigor of green seedlings, continue growth later in the season and are more susceptible to injury by early fall frosts. This recalls the frost susceptibility of chlorotic redwood shoots reported by Peirce (*17*) and further suggests a relation between chlorosis and low osmotic pressure due to failure to make sugar, as in wilting. Decreased winter loss as a result of a treatment which controlled the chlorosis is shown by the data in Table I. Seedlings chlorotic during their first or second year start growth tardily or not at all the following season. The number of dwarfed chlorotic plants which die during the summer is increasingly great during the second and third years in the seed bed. In transplanting, chlorotic seedlings are discarded.

[1] Determined by the indirect method of Briggs and Shantz in the Laboratory of Biophysical Investigations, Bureau of Plant Industry, United States Department of Agriculture.

TABLE I.—*Effect of iron-sulphate spraying on mortality of western yellow pine seedlings*

Series and plot.	Age of stock during treatment period.	Number of seedlings per square foot.	Treatments.			Dead seedlings.		
			Dates.	Strength of solution (grams per 100 cc.).	Amount $FeSO_4$ per square foot of bed.	Sept. 3 to Oct. 22, 1917.	Oct. 22, 1917, to Apr. 23, 1918. (winter-killed).	Sept. 3, 1917, to Apr. 23, 1918.
	Months.		*1917.*		*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*
I, treated	14 to 18	108	Aug. 2, 24, Sept. 3, 12, 22, and Oct. 2.	2	[a] 0.95	4	0	4
II, treated	..do....	75		1	[a] .47	5	0	5
I, control	..do....	115	.....	.....	.....	3	8	11
II, control	..do....	80	.....	.....	.....	4	15	19
III, treated	2 to 5	52	Sept. 3 12, 22, and Oct. 2.	2	[a] .95	13	0	13
Do	..do....	58		1	[a] .47	14	0	14
Do	..do....	63		.5	[a] .24	14	11	24
III, control	..do....	76	.....	.....	.....	14	9	22

[a] 0.1 pint of solution per square foot, equivalent to 0.02 inch of rain, applied to each treatment.

The prevalence of chlorosis in the Pocatello Nursery during September, 1917, was determined by examining several thousand plants of the different age classes of western yellow pine and Douglas fir. Of the first- and second-year western yellow pine seedlings 82 and 62 per cent, respectively, were chlorotic; while 74 per cent of the transplants grown two years in the seed bed and one year in the transplant bed were chlorotic. First-, second-, and third-year seedlings of Douglas fir were chlorotic to the extent of 6, 65, and 26 per cent, respectively; while 15 and 62 per cent of the transplants grown three years in the seed bed and one and two years, respectively, in the transplant bed were chlorotic.

EFFECT OF WATERING

It was at first thought that too heavy watering might have been responsible for the chlorosis at the Pocatello Nursery. While an examination of the condition of the soil did not indicate water-logging, variations in the amount of artificial watering were tested. Four plots of Douglas fir seedlings approximately 2 months old were given varying amounts of water throughout a period of slightly over two months. The results appear in Table II. The artificial watering was at first given approximately once a week and amounted to the equivalent of 0.55 inch of rain on plot D, the most heavily watered plot. Plot C received two-thirds of this amount, plot B one-third, and plot A none. After the first month the amount of water added at each watering was decreased because of the difficulty of avoiding run-off, and the frequency of application was increased. The plots in this experiment were free from chlorosis at the beginning of the period, and all of them later exhibited more or less yellowing. The amount of water applied artificially, combined with the natural precipitation, did not total an ex-

cessive amount, except possibly in plot D. This experiment was carried on in a section of the nursery in which the disease did not prove to be prevalent, and little chlorosis occurred in any of the plots. The entire number of yellow seedlings shows an increase with increased watering through all four plots for the last three counts and a somewhat less marked but similar relation for the earlier counts. The magnitude of the difference is, however, not sufficient to permit positive conclusions. Whether the apparent effect of the watering in increasing chlorosis was mostly due to the solutes in the excess water, to cooling the soil, or to hindering aeration, it is not possible to say. That the entire effect of the watering should have been due to disturbance of aeration, or temperature, seems scarcely possible in the cases of plots B and C, which received relatively little artificial watering. These plots did not seem excessively wet, but the soil of plot D was sufficiently wet to permit the development of moss—abnormally wet for this nursery.

TABLE II.—*Effects of different amounts of artificial watering on chlorosis in 4- to 5-month-old Douglas fir seedlings*

	Plot A, unwatered.[b]	Plot B, lightly watered.[b]	Plot C, moderately watered.[b]	Plot D heavily watered.[b]
1917.				
Rainfall and artificial watering (in inches):				
First half of August	Traces.	0.54 (3)	1.11 (3)	1.65 (3)
Last half of August	0.25 (3)	.34 (4)	.43 (4)	.53 (4)
First half of September	1.07 (6)	1.34 (7)	1.61 (7)	1.91 (7)
Last half of September	.71 (2)	1.07 (6)	1.43 (6)	1.83 (6)
First half of October		.18 (2)	.36 (2)	.56 (2)
Total, Aug. 2 to Oct. 6	2.03 (11)	3.47 (22)	4.94 (22)	6.48 (22)
Percentage of seedlings found chlorotic:[a]				
Sept. 1	7.8	11.8	10.7	17.9
Sept. 13	6.1	7.5	7.0	14.7
Sept. 22	1.7	4.3	5.1	8.4
Oct. 2	.4	2.2	2.9	4.8
Oct. 22	.4	2.2	2.6	4.0

[a] Two square feet counted in each plot. Number of seedlings per square foot at beginning of test: Plot A, 241; B, 244; C, 363; D, 278.
[b] Figures in parenthesis indicate total number of days on which rain or artificial watering occurred.

A pathologic condition may be encountered in certain conifers growing in wet situations. This condition would be unfavorable and therefore would result in subnormal vigor and growth of the plants subjected to such abnormal conditions. In studying hypertrophied lenticels at the Bessey Nursery, near Halsey, Nebr., one of the writers (*8*) conducted an experiment in heavy watering, in which irrigations approximately equivalent to 2.2 inches of rainfall were repeated 17 times during a period of three months on western yellow pine transplants grown two years in the seed bed and one year in the transplant bed. Considerable chlorosis appeared in the heavily watered beds, the plants of which were originally thrifty and free of chlorosis, while the controls remained nonchlorotic. There is also a possibility of a lack of proper aeration of the soil and of

oxygen hunger as a very probable and effective stimulus in inducing chlorosis in a mesophyte like Douglas fir in an excessively wet soil.

SPRAYING WITH FERROUS SULPHATE

Spraying with ferrous sulphate was tested on western yellow pine and Douglas fir. The first tests were on seedlings of the former species approximately 14 months old. Plots 4 by 10 feet were laid out, series I in beds in which chlorosis was not serious, and series II in beds in which it was very prevalent. The two plots in each were adjacent and parallel. Care was taken to choose plots as nearly as possible identical in vigor, number of seedlings per square foot, and amount of chlorosis. One of the plots in each series was sprayed with iron-sulphate solution at the rate of 2 gm. of sulphate per 100 cc. of water, and the other was given an

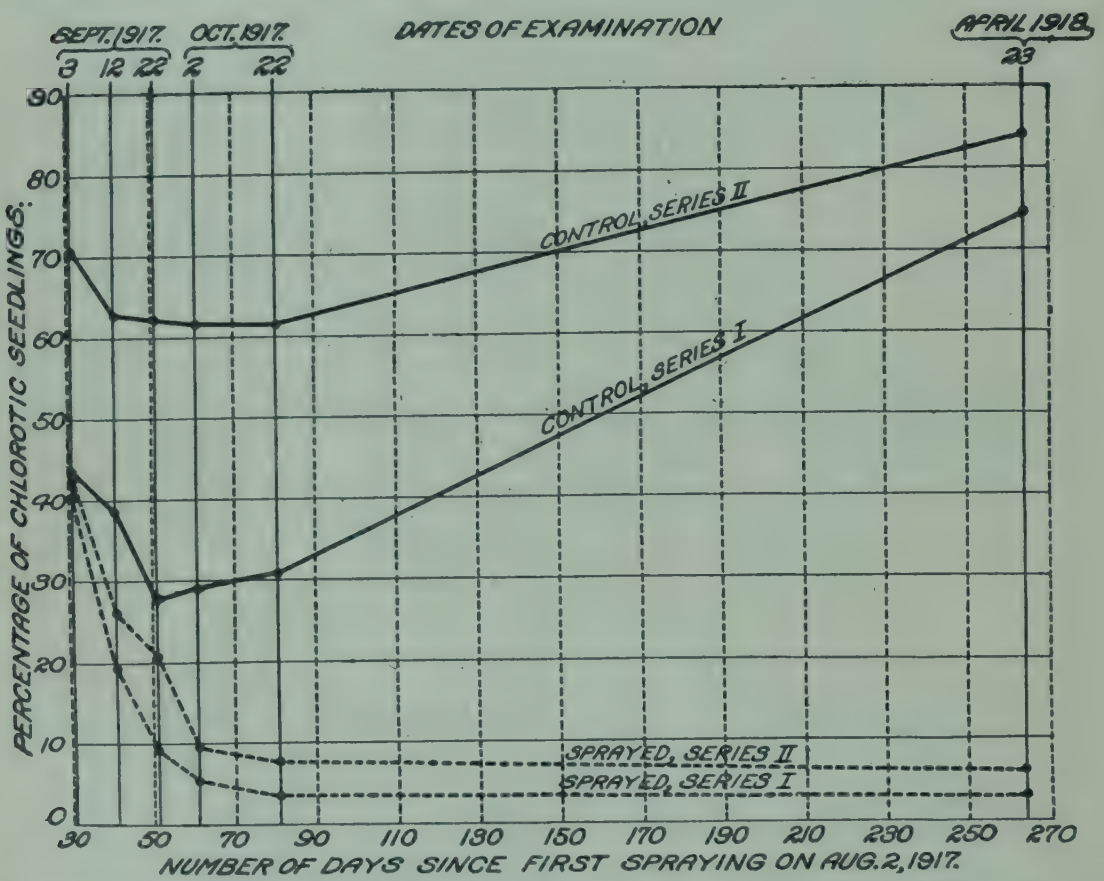
DATES OF EXAMINATION
SEPT. 1917.
OCT. 1917.
APRIL 1918.
3 12 22 2 22 23
PERCENTAGE OF CHLOROTIC SEEDLINGS.
0 10 20 30 40 50 60 70 80 90
CONTROL, SERIES II
CONTROL, SERIES I
SPRAYED, SERIES II
SPRAYED, SERIES I
30 50 70 90 110 130 150 170 190 210 230 250 270
NUMBER OF DAYS SINCE FIRST SPRAYING ON AUG. 2, 1917.

FIG. 1.—Graph showing the effect of a ferrous sulphate spray treatment on chlorosis in seedlings of western yellow pine 14 to 18 months old.

equal quantity of water only and was used as a control. The spraying was done with a hand-spray pump and was begun on August 2, 1917. In each case the plot selected for the treatment appeared slightly more chlorotic than the control at the time of the first treatment. On August 24, after two sprayings, it was evident that chlorosis had been decreased but that chemical injury to the youngest growth had resulted from the treatment. This injury is somewhat surprising, in view of the successful use of 8 per cent solutions on pineapple (*12*). The difference in results may, of course, be due to difference in the localization of the solution on

the surfaces of the two plants. Conifers are very difficult to coat evenly with a spray. Also the fact that there had been practically no rain from the first treatment to the time the injury was observed may have been responsible for the degree of injury by the 2 per cent solution. In a region of heavier rainfall this solution, or even a stronger one, might be entirely harmless to conifers. The treatments were continued, but with a 1 per cent solution at the rate of only 0.1 of a pint, containing approximately 0.47 gm. of ferrous sulphate per square foot of seed bed. Sprayings with this weaker solution were made on August 24, September 3, September 12, September 22, and October 2; and the seedlings on sample areas were counted and classified as to the degree of chlorosis on different dates in September and October, and again in April of the following year.

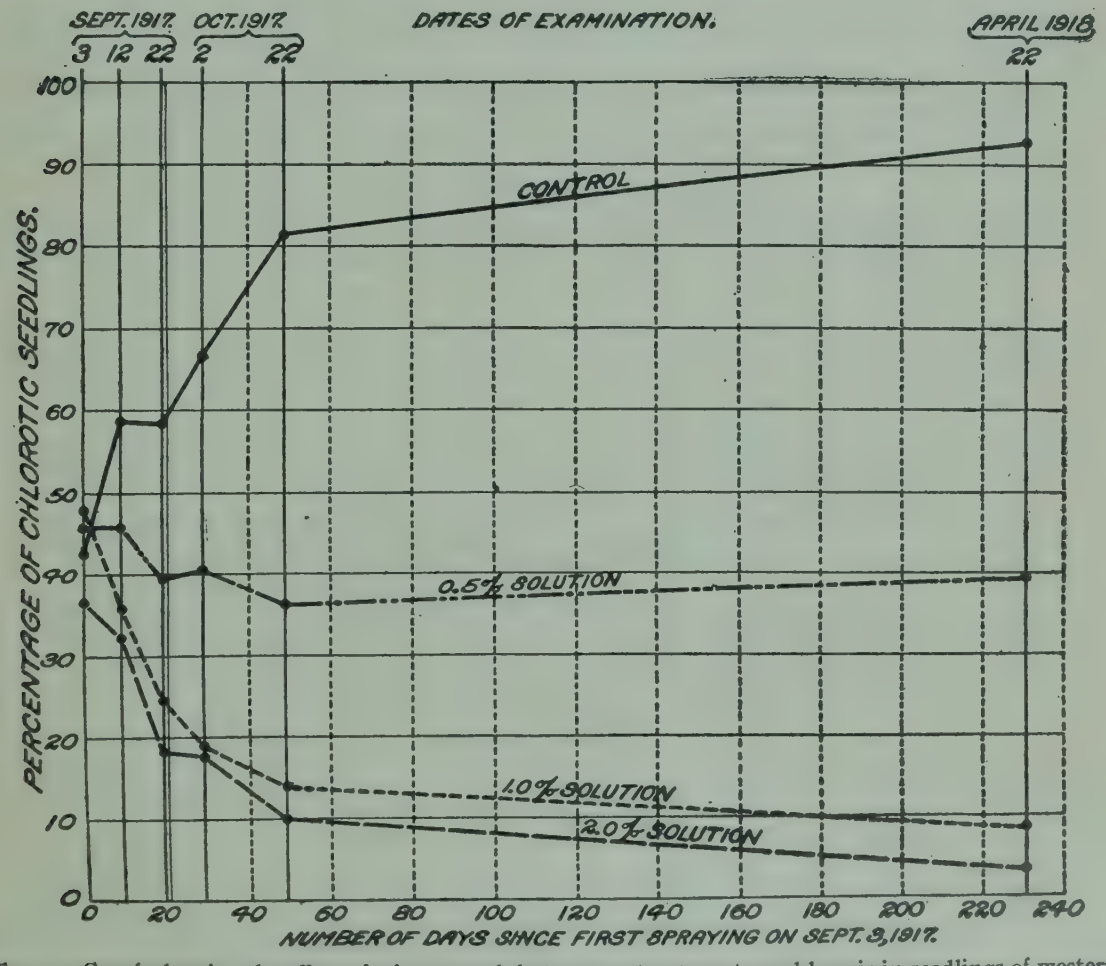

FIG. 2.—Graph showing the effect of a ferrous sulphate spray treatment on chlorosis in seedlings of western yellow pine 2 to 5 months old.

The results are shown graphically in figure 1. Decided inprovement in the color of the sprayed plots during the period covered by the counts is indicated by the data. The undiminished persistence of the good effect through the winter, a total of 6½ months after the last spraying, and the smaller percentage of winterkilled seedlings in the sprayed plots (Table I) are worthy of note.

At the time the first counts were made on the older seedlings (September 3) plots of the same size were also laid out in beds of both western yellow pine and Douglas fir of the current year's sowing and were therefore

about 3 months old. The results with the young western yellow pine (fig. 2) are more striking than those with the older stock. Autumn losses, presumably due to late damping-off, were not affected by the treatments; but winterkilling was entirely prevented (Table I). The heaviest treatment seemed to give better results than the lighter ones, so far as correcting chlorosis was concerned, both at the fall and the succeeding spring examinations, but resulted after the third treatment in the blackening of some of the leaves. The chemical injury was even more marked at the time of the spring examination, when practically every seedling in all the western yellow pine plots treated with the 2 per cent solution showed chemical injury, whereas the plots treated with the weaker solution showed none.

With young Douglas fir (fig. 3) the amount of chlorosis initially present was less, and the untreated seedlings as well as the treated improved in color during the course of the experiment. In so far as chlorosis is corrected, the results are similar to those secured with western yellow

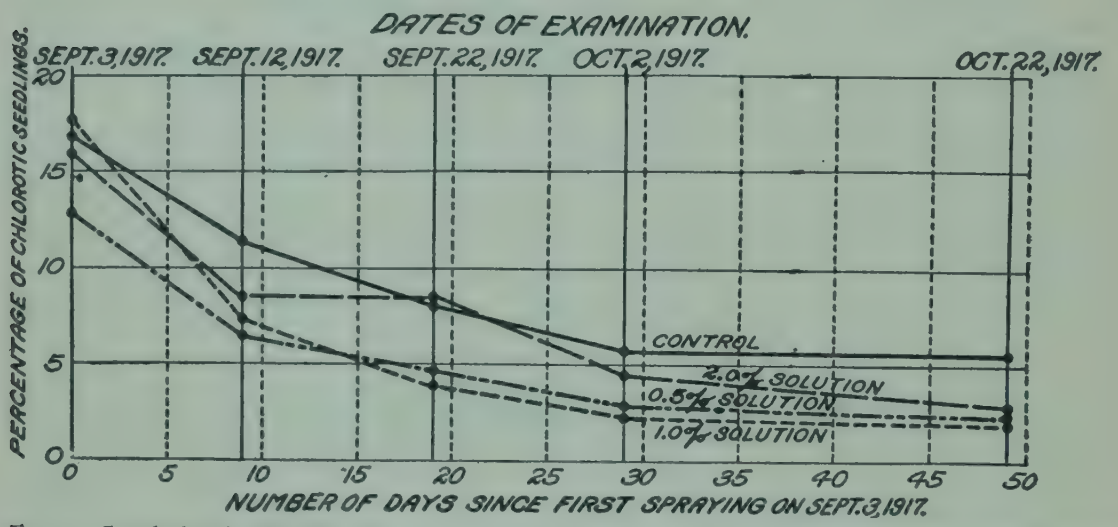

FIG. 3.—Graph showing the effect of a ferrous sulphate spray treatment on chlorosis in seedlings of Douglas fir 2 to 5 months old.

pine. On Douglas fir, however, the heaviest treatment was no more effective against chlorosis than the lightest; the intermediate gave the best results. In view of this and the injury to western yellow pine from the strongest solution, it appears that only the intermediate strength (1 per cent) should be used on conifers, at least if repeated spraying is practiced.

Though the total area counted in all the spraying experiments with first-year seedlings was small—12 square feet in the treated plots and 6 square feet in the controls—the data obtained from the counts show on the whole such consistent and decided improvement in the sprayed plots as to leave no reasonable doubt about the therapeutic value of the treatment for western yellow pine. Observations on the entire area of the western yellow pine experimental plots (200 square feet treated and 120 square feet in the controls) indicate that the sample areas on which the counts were made were reasonably representative of the entire plots.

The contrast between the treated and untreated plots of western yellow pine at the close of the experiments was very strong throughout.

No attempt was made to exclude the ferrous sulphate from the roots. In view of the high absorptive capacity for iron sulphate of the calcareous soil with which Sachs worked (*19*, *20*) and the prompt reaction (fig. 2) following the small amount of the sulphate added by the writers in the 1 per cent solution treatments on the younger western yellow pine seedlings (fig. 2 and Table I), it is believed that the effect of the iron-sulphate spraying was due to the entrance of traces of iron into the leaves, presumably mostly through the stomata, though Molisch (*14*) reports it as entering through the cuticle.

Forest officers report that 1 per cent ferrous sulphate sprayings begun in April at the Morton Nursery corrected chlorosis in 2-year-old seedlings of both jack pine (*Pinus banksiana*) and western yellow pine by June. Scotch pine did not show chlorosis; jack pine showed it most. The control of the yellowing was not absolute but was practically complete by the end of July. The iron-sulphate spray treatment is considered so successful that it has now been put into general use on all the jack pine and western yellow pine seed beds at the Morton Nursery.

VALUE OF THE EXPERIMENTS

It appears from the literature cited in the introduction that on soils containing considerable calcium carbonate there often occurs a chlorosis which can be corrected by the addition of iron in soluble form to either the roots or the leaves. The trouble-making capacity of the calcium carbonate, though not always in evidence, appears to be more or less specific. Other calcium salts and other carbonates do not seem equally effective as causes of chlorosis. It is reasonable to suppose, in view, among other things, of the precipitation of iron in alkaline solutions, the apparent substitution of iron for calcium in soil (*15*), and the nonavailability of colloidal iron (*7*, *11*) that the trouble was chiefly due to the lack of dissolved iron in the water of certain calcareous soils. However, in the lime soil it might conceivably be that the balance of the solution for plants which are not distinctly calciphile is so disturbed as to make more than the usual amount of iron necessary to maintain the plants in normal health on such soils. A further complication is the fact that the distribution of chlorosis in different parts of the same plant is sometimes such as to indicate that at least part of the difficulty may be due to derangements in conduction instead of or in addition to absorption failures. Furthermore, physiologists are not all ready to agree that the lack of green is really a symptom of a specific iron hunger, even in cases in which the remedial value of iron addition is demonstrated. The writers' results have made no addition to the knowledge of the immediate cause of the chlorosis or the way in which the addition of iron works in correcting it. These complications are mentioned merely to show that fundamental

work on chlorosis lies in the domain of the biochemist rather than of the pathologist or the forester.

The immediately practical applications are fortunately simpler. The writers have added three gymnosperms to the considerable list of angiosperms in which chlorosis can be relieved by spraying ferrous sulphate on the surfaces of the leaves. While the best way to avoid chlorosis in coniferous nurseries is probably to avoid soils containing any considerable quantities of calcium carbonate, an entirely practicable method of treatment is offered by which chlorosis can apparently be relieved in coniferous nurseries on lime soils. At the rate at which the experimental spraying was done, using the 1 per cent solution, which on the whole gave the best results, 1 pound of the relatively cheap ferrous sulphate is sufficient for over 900 square feet of bed. While with larger stock more material would be required, the process would still be relatively cheap. Johnson (*10*), using a solution eight times as strong, reported the total cost of spraying pineapples as $0.60 per acre for each spraying. In a business as intensive as that of raising coniferous nursery stock such a cost item would be almost negligible.

RELATION BETWEEN CHLOROSIS AND GROWTH

Observations through several seasons at the Pocatello Nursery have indicated a relation between chlorosis and poor growth. In order to secure data on this relationship the seedlings in the control plots whose counts are given in figure 1 were classified by their apparent vigor of growth as well as according to their chlorotic condition. The counts showed for the first series that 23 per cent of the seedlings classed as vigorous were chlorotic, while 42 per cent of the weak seedlings were in the chlorotic class. For the second series the difference was about the same, 46 per cent of the vigorous seedlings being chlorotic against the very high proportion of 73 per cent among the seedlings classed as weak. In an effort to put this relationship on a more exact basis, specimens were selected from each class and subclass of seedlings of which a sufficient number were available to give a reasonable numerical basis, and measurements of roots, stems, and leaves were made.

METHODS OF SECURING MEASUREMENTS

The seedlings taken were selected by a process of mechanical elimination, every fifth seedling being chosen in most of the cases, so that they are believed to be representative of the groups from which they came. The leaf surface values were obtained by a method which does not pretend to give the absolute surface accurately, but which is believed to give sufficiently accurate relative values to permit a comparison of the different groups of plants.

The surfaces of the primary and secondary leaves of western yellow pine were determined separately on account of their different shapes.

The primary leaves are approximately semicircular in cross section, and for the purpose of obtaining a comparative surface value were considered as halves of cylinders having a radius equal to the thickness of the leaf midway between the base and the tip, with the length equal to the length of the leaf. The perimeter of the cross section at this point was taken as $\pi R + 2R$—that is, the sum of the lengths of the curved and the flat margins of the cross section. The surface of the leaf was taken as $S = L(\pi R + 2R)$, in which L equals the length of the leaf. A sufficient number of primary and secondary leaves from each plant were measured to allow averaging (usually from 25 to 100, depending on the number per plant). The total primary leaf surface for the plant was obtained by multiplying the surface of the average leaf by the entire number of primary leaves. The secondary leaves were in most cases in fascicles of three, and their cross-sectional shape may be diagrammed as in figure 4. The same assumptions were made in this case as in the case of the primary leaves, the leaf being taken as an exact third of a cylinder with the radius equal to the thickness of the leaf midway between the base and the tip, and the surface calculated by the formula $S = L\left(\frac{2\pi R}{3} + 2R\right)$. This formula appears to be more nearly correct than a paraboloid formula, and it is believed that it offers a better basis for comparing the leaf surface of one group of plants with that of another than would be given by statements of the average number, length, breadth, and thickness of the leaves.

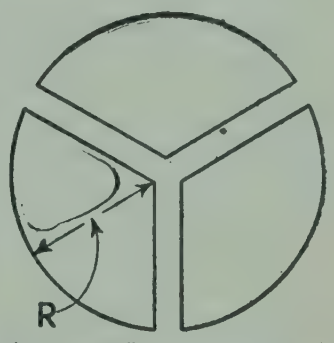

FIG. 4.—Cross-sectional shape of fascicle of three secondary leaves of western yellow pine.

INTERPRETATION OF THE MEASUREMENTS

From Tables III and IV it appears that the height and the weight of the tops, the length and the weight of the roots, the diameter of the stems at the root collar, the length and thickness of the secondary leaves, and the average total leaf surface of the plants was less for chlorotic plants than for green seedlings of the same vigor class and that terminal bud formation was most common and most pronounced in the most vigorous plants. The data indicate that the failure to form buds is related to a general lack of vigor, which in many cases is associated with chlorosis. The adverse effects of chlorosis on terminal bud formation and development is significant in connection with the high winter mortality of the strongly chlorotic seedlings.

The small size of the different parts of the plants in the chlorotic seedlings, as compared with the green seedlings, is, on the whole, fairly uniform. Two exceptions to this are, however, noteworthy. In Table III it appears that the roots of the chlorotic plants are nearly as long

as those of the green plants. However, the weight of the roots was decidedly less in the chlorotic than in the green plants. This agrees with the field observations, indicating an association between chlorosis and a deficiency of fibrous lateral roots. It will be noted that the strongly chlorotic plants in the vigorous class had a root weight considerably less than that of the green plants in the weaker class. While the roots of these plants were also somewhat shorter, the weight difference was distinctly greater.

The other lack of parallelism between increased chlorosis and decreased growth is in the primary leaves. These were nearly as well developed in the chlorotic plants as in the green plants. This is considered significant as indicating that the plants which were decidedly chlorotic and lacking in vigor during the second season were not originally very different in their growth rate from the others. This relationship is most easily seen in the three columns under "Relative leaf surface" in Table IV.

TABLE III.—*Root, top, and terminal bud development of the different type classes of 2-year-old seedlings*

UNTREATED WESTERN YELLOW PINE [a]

Type classes.	Number of seedlings.	Average diameter of stem at root collar.	Tops.		Roots.		Average total weight of seedlings.	Terminal buds.		
			Average length.	Average weight.	Average length.	Average weight.		Percentage of seedlings forming terminal buds.	Average length.	Average diameter.
		Inches.	*Inches.*	*Gm.*	*Inches.*	*Gm.*	*Gm.*		*Inches.*	*Inches.*
Vigorous, green	100	0.075	2.2	1.40	11.7	0.57	1.97	9	0.33	0.16
Vigorous, slightly chlorotic	95	.065	1.7	.77	9.7	.33	1.11	2	.23	.12
Vigorous, strongly chlorotic	22	.062	1.6	.52	8.6	.20	.72	0	.....	.....
Weak, green	100	.060	1.6	.54	9.6	.26	.80	0	.....	.....
Weak, slightly chlorotic	100	.058	1.4	.35	9.3	.18	.52	0	.....	.....
Weak, strongly chlorotic	100	.045	1.2	.22	9.6	.12	.34	0	.....	.....
UNTREATED DOUGLAS FIR [a]										
Green	50	.046	2.8	.34	9.6	.15	.50	100	.15	.08
Chlorotic	50	.038	2.3	.22	8.2	.08	.30	86	.10	.04

[a] The number of chlorotic plants in the treated plots at the end of the season was insufficient to serve as a basis for measurement.

TABLE IV.—*Leaf development of the different type classes of 2-year-old seedlings*

UNTREATED WESTERN YELLOW PINE [a]

Type class.	Number of seedlings.	Primary leaves.					Secondary leaves.				Average total leaf surface per plant.	Relative leaf surface.[b]		
		Average number per plant.	Average length.	Average thickness.	Average leaf surface per plant.	Percentage of seedlings bearing secondary leaves.	Average number per plant.	Average length.	Average thickness.	Average leaf surface per plant.		Primary leaves.	Secondary leaves.	Total.
			Inches.	*Inches.*	*Square inches.*			*Inches.*	*Inches.*	*Square inches.*	*Square inches.*			
Vigorous, green	100	37.7	1.05	0.016	3.27	100	32.1	2.61	0.023	7.89	11.15	100	100	100
Vigorous, slightly chlorotic	100	40.8	.80	.012	2.02	100	29.9	1.82	.015	3.34	5.36	62	42	48
Vigorous, strongly chlorotic	22	42.2	.83	.014	2.52	100	25.2	1.63	.015	2.52	5.05	77	32	45
Weak, green	100	31.2	.85	.013	1.77	99	23.4	1.45	.015	2.08	3.86	54	26	36
Weak, slightly chlorotic	102	46.4	.67	.013	2.08	68	18.1	1.09	.017	1.37	3.45	64	17	31
Weak, strongly chlorotic	100	38.1	.60	.013	1.41	43	13.7	1.05	.015	.88	2.29	43	11	21

UNTREATED DOUGLAS FIR [a]

Green	50	95.1	0.50	0.010	2.44	.....	.....	.....	.....	.....	2.44	100	.....	100
Chlorotic	50	83.0	.45	.007	1.34	.....	.....	.....	.....	.....	1.34	55	.....	55

[a] The number of chlorotic plants in the treated plots at the end of the season was insufficient to serve as a basis for measurements.
[b] Untreated "vigorous green" leaves taken as 100.

This difference between the first and second season's growth of the same plants is thought to have some bearing on the character of the association between chlorosis and lack of vigor. Three possible explanations of this association present themselves: (1) A general lack of vigor may predispose to chlorosis; (2) the conditions which cause reduced vigor at this nursery may also favor chlorosis, so that the two phenomena are coordinate effects of the same set of conditions; (3) the poor growth of chlorotic plants may be a result of the chlorotic condition.

The first of these possibilities has little to recommend it from the theoretical standpoint, in so far as vigor is judged by the growth rate of the tops. Sachs (*19*) finds iron-hunger chlorosis especially common in rapidly growing shoots. The evidence in Tables III and IV that the chlorotic seedlings were not seriously deficient in growth of tops at the beginning of their second season tends further to discredit this first suggested explanation. Such difference in the primary leaf system as did exist between the different subclasses was more likely the effect of chlorosis during the preceding year than the cause of chlorosis during the current season. However, the root weights are of some interest. That poor root development may predispose to chlorosis is entirely probable. In a soil deficient in available iron, a plant with little root surface would presumably be especially liable to iron hunger.

The second and third explanations are both believed to apply in part to the obvious association of chlorosis and poor development. Lack of balance of soil solution around the roots of the plant sufficient to interfere seriously with the chlorophyll-forming function might easily interfere more or less with some of the other processes in the plant to an extent sufficient to decrease growth rate. Furthermore, the shortage of chlorophyll and consequent decreased ability to synthesize carbohydrates would very naturally result in decreased growth of at least some parts of the plant. It is, therefore, the belief of the writers that poor development of roots (but not of tops) is probably a contributory cause of chlorosis, that both chlorosis and poor development of the whole plant may in part be parallel and independent results of excessive lime, and that chlorosis is almost certain to result in a still further decrease in growth. A circular relation, therefore, seems to obtain.

The data on Douglas fir in Tables III and IV indicate that with this species, as with the western yellow pine, there is a decided difference in growth between green and chlorotic seedlings. With this species as with the other, the weight of the roots of the chlorotic seedlings is much more deficient than the length. From the data it is not possible to determine separately the first and second year's growth, as was possible to a certain extent in the western yellow pine. The deficient terminal bud production and development on the part of the chlorotic plants is as evident in Douglas fir as in western yellow pine.

A condition associated with chlorosis in Douglas fir which does not appear in the measurements is its abnormally prompt loss of turgor on the cutting off of the water supply. The leaves of chlorotic seedlings wilted so quickly after the plants were taken up that measurements of the width of the leaves could not have been accurately made for this species. Another difference not shown by the measurements is in the color of the terminal buds formed. The terminal buds of normal Douglas fir are a reddish brown, while those of the chlorotic seedlings vary from a light brown to a brown.

No relation between the growth rate and the artificial supply of iron was evident from an examination of the beds. As the treatments were not begun until the latter part of the growing season, no material effect was to be expected.

SUMMARY

Chlorosis has been the most serious problem encountered in the successful production of coniferous nursery stock at a nursery in southern Idaho. The disease affects all coniferous species grown in this nursery. With chlorosis were associated poor growth of roots, stems, and leaves, failure to form normal terminal buds, and susceptibility to winter injury.

The importance of excessive soil moisture as a cause of chlorosis has not been definitely determined. Preliminary experiments indicate, however, that it is relatively unimportant.

Chlorosis in western yellow pine at the Pocatello Nursery has been definitely corrected by spraying with ferrous sulphate at 10-day intervals. Similar, though less decisive, results were obtained with Douglas fir. A 1 per cent solution in amounts sufficient to wet the tops thoroughly proved the most satisfactory treatment. A 2 per cent solution ultimately caused chemical injury to practically all the plants. In a region of more frequent rains the stronger solution might be better.

The control of chlorosis in jack pine and western yellow pine at the Morton Nursery in Nebraska by spraying with a 1 per cent solution of ferrous sulphate has given such evidence of success that it has been adopted as a part of the regular nursery practice.

The three soils on which conifers have been found decidedly chlorotic all contain considerable amounts of carbonate and have been formed, in part at least, from limestone. The nursery water supply at Pocatello also contains much calcium bicarbonate. No definite correlation could be found between chlorosis and the amounts of calcium or of carbonate obtained by hydrochloric-acid digestion analysis.

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ROOTING STEMS IN TIMOTHY

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INTRODUCTION

In botanical and agricultural literature there are many references to stolons and rootstocks or rhizomes in timothy. However, the statements made and the terms employed are so confused that it is scarcely possible to determine in any case the specific nature of the phenomenon that occurs. It is by no means clear whether rooting stems above or below ground are referred to; neither is it clear to what extent and under what conditions such stems are actually formed. Some writers have concluded that stolons and rootstocks are varietal characteristics, while others indicate that they are very generally found in timothy. No effort has been made by the writers to find the first mention of stolons or rhizomes in the literature on timothy, but apparently they were not mentioned by botanists or agronomists prior to the time of Linnaeus. In the literature of the nineteenth century, however, there are many references, principally to what are termed stolons; but no detailed information is given regarding them, and consequently there is a very general lack of knowledge of just what they really are, or as a matter of fact that they actually exist.

In connection with their timothy breeding investigations, the writers have had the opportunity to study the timothy plant closely, and they have devoted some attention to the rooting stems, which it sometimes produces. The results of these studies it is hoped will clear up the subject somewhat. Most of the data upon which this paper is based were obtained at the Timothy Breeding Station, which is conducted cooperatively by the United States Department of Agriculture and the Ohio Agricultural Experiment Station, at Elyria, Ohio.

TWO KINDS OF UNDERGROUND ROOTING STEMS

In June, 1918, some timothy plants were found growing in a field where timothy stubble had been plowed under the previous fall. These plants on casual observation had the appearance of plants which had grown from seed, but a close examination disclosed the fact that many of them were attached by underground stems to the stubble of the old plants that were turned under. The finding of these underground stems led to further search for plants possessing them and to critical studies regarding their formation. Abundant material was available at the station in the fall of 1918 and in the summer and fall of 1919 in

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fields where timothy stubble and plants had been plowed under at different times of the year.

From their investigations the writers found that underground rooting stems in timothy are of at least two kinds and that each develops in a different way. One type of underground stem is that illustrated in Plate 39, A. This type develops frequently when timothy stubble or plants are plowed under in the summer or early fall. It is the normal thing in timothy for the bud that forms the new shoot to develop from a node below one of the enlarged internodes at the base of an old culm. This is the way the timothy plant reproduces itself vegetatively. During the early growth of the shoot the nodes from which the leaves arise are close together. When the shoot grows in length to form a culm with a spike, only about six of the upper internodes elongate. The total length of the 10 to 20 unelongated internodes at the base of the culm ordinarily does not exceed 0.4 inch. Sometimes, however, it is somewhat longer. From the basal nodes, the root system of the new plant is produced.

While the foregoing is what takes place normally, the development of the new shoots may be modified appreciably by external conditions. When timothy stubble or plants that have passed the seedling stage are plowed under, or are similarly covered with soil, buds that make the new shoots start development in the normal way, usually only one bud on a stem. But the young shoot, instead of rooting where it is formed, almost immediately adjacent the mother plant, grows toward the light. In doing so the internodes at or near the base of the new shoot, sometimes only one, usually two or more, elongate, thus pushing it up to the surface in a nearly vertical direction. In many cases small fibrous roots develop from the nodes between these elongated basal internodes. It is in this way that undergound stems of rootstock-like appearance are formed. The length of the stem produced by the elongation of the basal internodes and the number of internodes involved depend to a very large extent on the depth to which the old culms are covered with soil. Some have been found having eight internodes, and some with a total length of 5.3 inches. Ordinarily these stems do not extend entirely up to the surface of the soil. An occasional shoot has been observed with its crown 3 inches below the surface, although the crowns are usually much nearer the surface than this. When produced under normal conditions, the crown of the young shoot is at the surface of the soil. The varying depth at which the crowns of the shoots attached to underground stems occur may be influenced by the extent of the leaf growth while it is still beneath the surface. The elongation of the basal internodes, together with the growth that takes place in the leaves, brings the tips of the leaves to the surface. The shoots with the greatest leaf development probably reach the surface with the least elongation of the basal internodes, other conditions being equal. This may explain

why some shoots have their crowns rarther beneath the surface than others, even though the buds from which they were produced were covered to the same depth with soil.

Root systems develop from the crown of all timothy shoots having underground stems, and each shoot is soon capable of continuing its growth independently of the mother plant. On a typical plant, selected in November, 1919, from a field in which timothy had been plowed under one year previously, the underground stem connecting the new plant with the parent plant was partly decayed and disintegrated, showing that the plant no longer had any vital connection with the stem from which it originated.

Underground stems of the same type can be induced by placing soil to a sufficient depth about the base of a growing timothy plant. An underground stem which has developed in this way is shown in Plate 39, B. The plant illustrated originated from a seed sown in the spring of 1919. In the following summer soil was placed about the plant to the point indicated by *x* to the right of the figure. The shoot on the left grew from a bud at the base of a culm of the parent plant. The underground stem that attached the new shoot to the mother plant was 1 inch long and was composed of a single elongated internode. The crown of the shoot was ½ inch below the surface of the soil which surrounded it.

The second type of underground stem is found where timothy plants with growing culms are covered with soil, as by plowing. Buds that sometimes form on the culms of these plants frequently develop into shoots and ultimately into independent plants, and the culms themselves become underground rooting stems. This type is illustrated in Plate 40, A. That the stem *a* had developed as a culm before it was plowed under is clearly shown by the lower elongated internode, which is enlarged in diameter and which forms what is commonly, but erroneously, termed a bulb. Further evidence that this stem had already developed as an ordinary culm before it was plowed under is found in the dried and partly disintegrated leaf at the second node above the enlarged basal internode. This leaf could hardly have grown to a length of several inches beneath the surface of the soil. In the plant illustrated in Plate 40, A, it was impossible to determine whether the shoot *b*, growing at the end of the stem, originated from the terminal bud of the culm or from a bud at one of the nodes farther back. It will be noted, however, that there is a secondary underground stem *c* arising from the second node above the enlarged basal internode. This is of the type shown in Plate 39. It is rarely that secondary shoots develop at nodes between the elongated internodes of timothy culms growing under ordinary conditions, but when the culms are covered with soil this frequently occurs. A rooting stem such as is shown in Plate 39 and in Plate 40, A, *c*, might have developed in connection with shoot *b* in Plate 40, A, had the culm *a* from which it arises been covered to a sufficient depth with soil.

A careful examination of the scattering timothy plants that appear in a field where a timothy sod has been plowed under leads to the conclusion that many if not most of them have not come from seed directly but have been produced vegetatively from the buried stubble or culms. This fact is important agronomically, especially when it is desired to grow pure strains of timothy for seed on the sod of a former timothy crop.

So far as the writers have been able to find, these two types of underground stems, both rooting or at least both capable of producing roots, are the only types of underground stems produced in timothy.

ABOVE-GROUND ROOTING STEMS

Rooting stems sometimes develop above ground, but they have rarely been observed at the Timothy Breeding Station. They are apparently formed when weak or decumbent stems come in contact with the soil. Such a stem is shown in Plate 40, B.

Regarding above-ground rooting stems, Witte [1] says (in translation):

> However, in a few cases of dwarf varieties I have noticed that the tufts were less firm, which in turn is due to the fact that the shoots grow out from the axils of the leaves situated higher up on the culm, as, for instance, from the second or third internode above the swollen one. Because in the case of certain varieties at least the lower part of the culm rests on the ground, these shoots easily take root, and in this way we get, as it were, a system of surface runners.

Since the rooting stems that form above ground are apparently but incidentally associated with a decumbent habit of growth, it is very doubtful if they should be regarded as a varietal or strain characteristic. Witte, however, has so regarded them. Ascherson and Graebner [2] have named and described a variety *stoloniferum*, but from their description it is not clear whether their variety includes plants with rooting stems above or below ground, or both. European literature contains so many references to rooting stems in timothy that it is probable there may be forms in Europe which are not found or are at least are not common in this country. On page 36 of his "Om Timotejen," [1] Witte figures a very spreading, short-culmed plant which closely resembles one or more of the forms regarded by some botanists as distinct species of Phleum, which are characterized in general by a decumbent, spreading habit of growth. The influence of these species or forms on timothy may explain why above-ground rooting stems are mentioned so frequently by European botanists and agriculturists. It is quite evident to the writers that underground stems are in no sense a varietal characteristic, since they can be induced without difficulty.

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[1] WITTE, Hernfrid. OM TIMOTEJEN, DESS HISTORIA, ODLING OCH FORMRIKEDOM SAMT OM FÖRÅDLINGSARBETENA MED DETTA VALLGRÄS PÅ SVALÖF. In Sveriges Utsädesför. Tidskr., Årgang 25, Häfte 1, p. 23–44; Häfte 4, p. 143–182; Häfte 5, p. 199–230, 24 fig. 1915. Résumé in German, p. 222–230. Bibliographical footnotes.

[2] ASCHERSON, Paul, and GRAEBNER, Paul. SYNOPSIS DER MITTELEUROPÄISCHEN FLORA. Bd. 2, Abt. 1, p. 143. Leipzig. 1898–1902.

NAMES NEEDED FOR DIFFERENT TYPES OF ROOTING STEMS

The question arises as to what name should be applied to rooting stems in timothy. Stems produced above ground are runners or stolons, according to a commonly accepted definition of these terms. However, the examples given in connection with the published definitions are by no means typical of the phenomenon that occurs in timothy. As for the underground rooting stems, those of the first type discussed in this paper are not essentially different from those found for example in Kentucky bluegrass and red fescue, except that they do not develop normally but are induced by unusual conditions. These stems are formed by the elongation of one or more of the lower internodes of the shoots which normally remain unelongated. The elongation of the internodes is for the purpose of placing the new shoots in a more favorable place for growth and development. This might be said to be the function of all underground stems, except possibly tubers, corms, and the like. But true rhizomes, by which are meant underground stems that root at the nodes and produce stems or leaves progressively, have in addition to this another definite function, that of reproduction. It would appear desirable, therefore, to designate by some specific name the underground rooting stems of timothy and other grasses that develop by the elongation of the basal internodes only under unusual conditions and for the purpose of placing the new shoot in a more advantageous location. Mr. C. V. Piper has suggested for such an organ the name "topothete," a Greek word from topos, a place or location, and thetu, meaning to place. It is evident from a study of the various terms applied to rooting stems and the definitions of these terms that those who formulated them did not appreciate sufficiently the fact that there are at least four distinct types of rooting stems produced by grasses and other plants, two kinds above ground and two kinds underground. Since the existing terms do not meet the needs of the case, the following names are proposed with the hope that they will be given favorable consideration.

DETERMINATE RHIZOME.—An underground stem which is disposed to root at the nodes and from which a single aerial shoot or tuft of shoots is produced; example, Kentucky bluegrass (*Poa pratensis* L.).

INDETERMINATE RHIZOME.—An underground stem, thickened or otherwise, which roots at the nodes and produces aerial shoots progressively; example, quack grass (*Agropyron repens* L.).

DETERMINATE STOLON.—An above-ground stem which roots at the nodes but does not produce aerial shoots progressively. Under this is included lax culms that have come in contact with the soil and have rooted at the nodes; examples, *Paspalum dilatatum* L. and crab grass (*Syntherisma sanguinalis* L.). This type of rooting stem is common in red top (*Agrostis alba* L.) and other species of Agrostis. These grasses, however, also have indeterminate stolons.

INDETERMINATE STOLON.—An above-ground stem which roots at the nodes and from which shoots are produced progressively; examples, Bermuda grass (*Cynodon dactylon* L.) and Rhodes grass (*Chloris gayana* L.).

It is quite probable that special terms may be needed where it appears desirable to make finer distinctions than are indicated above. However, it is thought that all of the rooting stems, especially those produced by grasses, can logically be designated by the general terms herein proposed. It is deemed advisable to discontinue the use of the term "rootstock," since this term is misleading and has been used by horticulturists in the sense of propagating stock. There appears to be no serious objections to the use of the word "runner" as a synonym of stolon, although it has been used in a more specific sense, as in the case of the strawberry.

SUMMARY

The references in literature to rooting stems in timothy fail to give a description of their nature or functions.

Investigations disclose two quite distinct types of underground rooting stems.

One type develops when the shoot that produces the new plant is covered with soil early in its growth. Some of the unelongated internodes connecting the shoot with the parent plant elongate, thereby pushing the shoot to the surface of the soil. Roots grow from the nodes between the elongated internodes.

A second type of underground rooting stem is produced when timothy plants with growing culms are covered with soil. Buds that sometimes form on the culms of these plants frequently develop into shoots and ultimately into independent plants. The culms then become underground rooting stems.

Many, if not most, of the scattering timothy plants that appear in a field where a timothy sod has been plowed under are produced vegetatively from buried stubbles or culms.

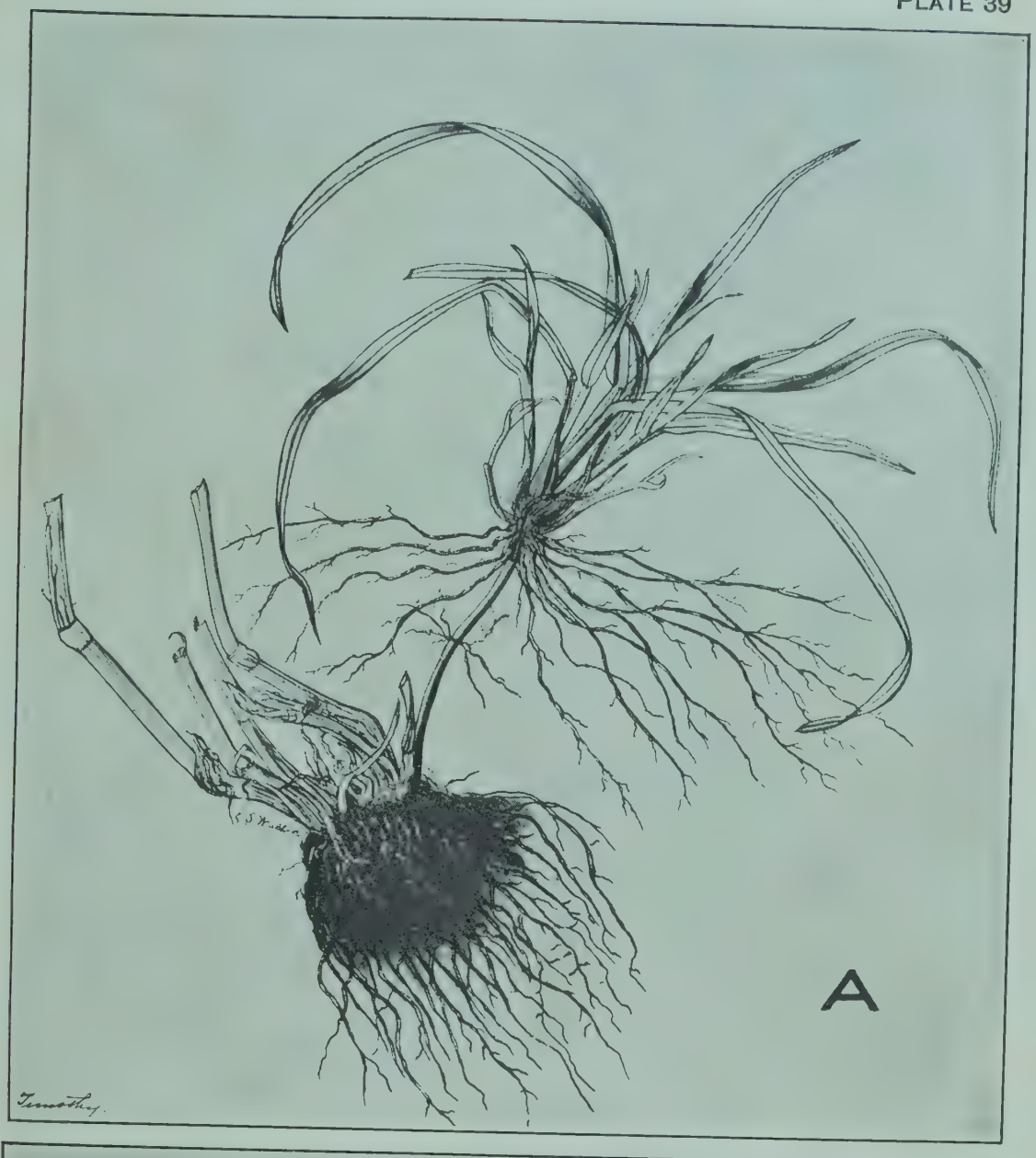
Above-ground rooting stems in timothy are not common in this country. Some botanists have regarded them as a varietal characteristic. It is very doubtful if they should be so regarded. Apparently they are formed most commonly when weak or decumbent stems come in contact with the soil.

Determinate and indeterminate rhizomes and determinate and indeterminate stolons are terms suggested to cover in a general way the types of rooting stems that are found especially in grasses.

PLATE 39

A.—A young timothy plant produced from a bud on a matured culm plowed under. It was pushed to the surface by a rooting stem formed by the elongation of two of its basal internodes.

B.—A rooting stem *b* induced by covering the parent plant *a* with soil to the point *x*.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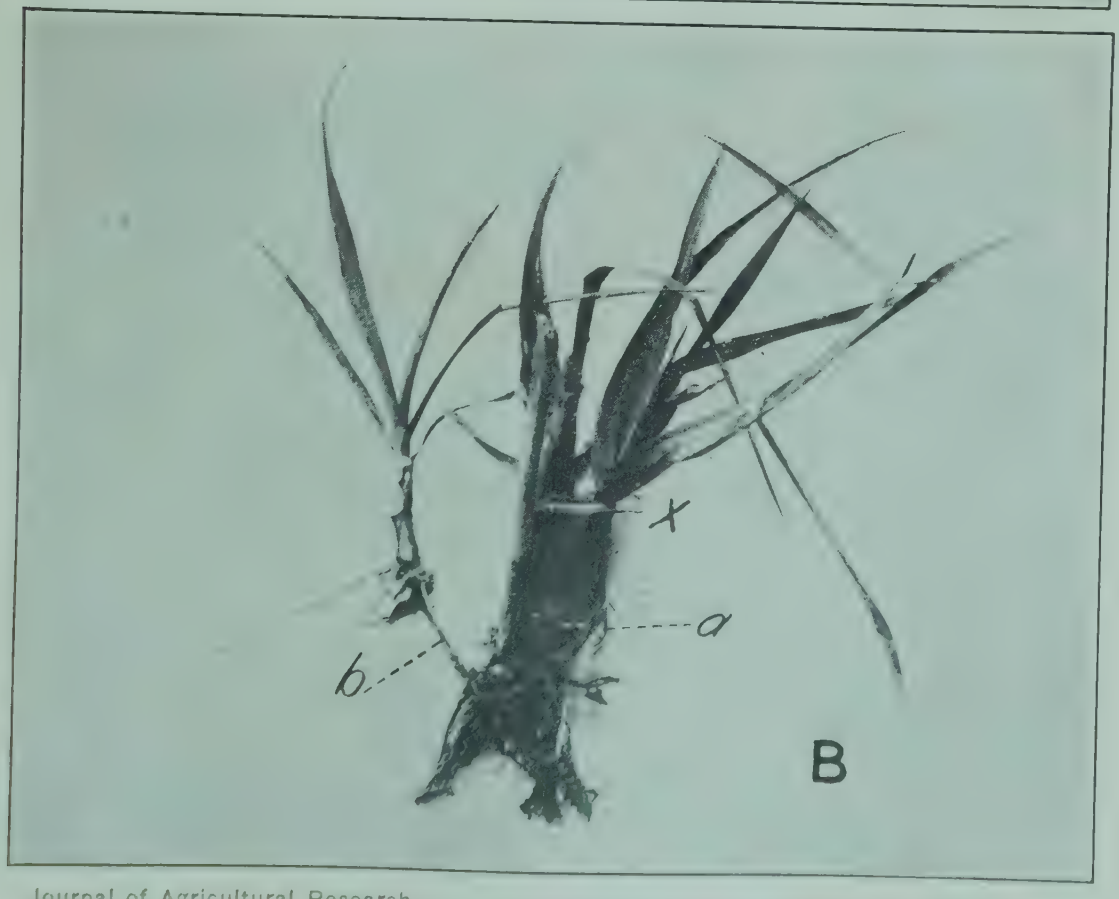
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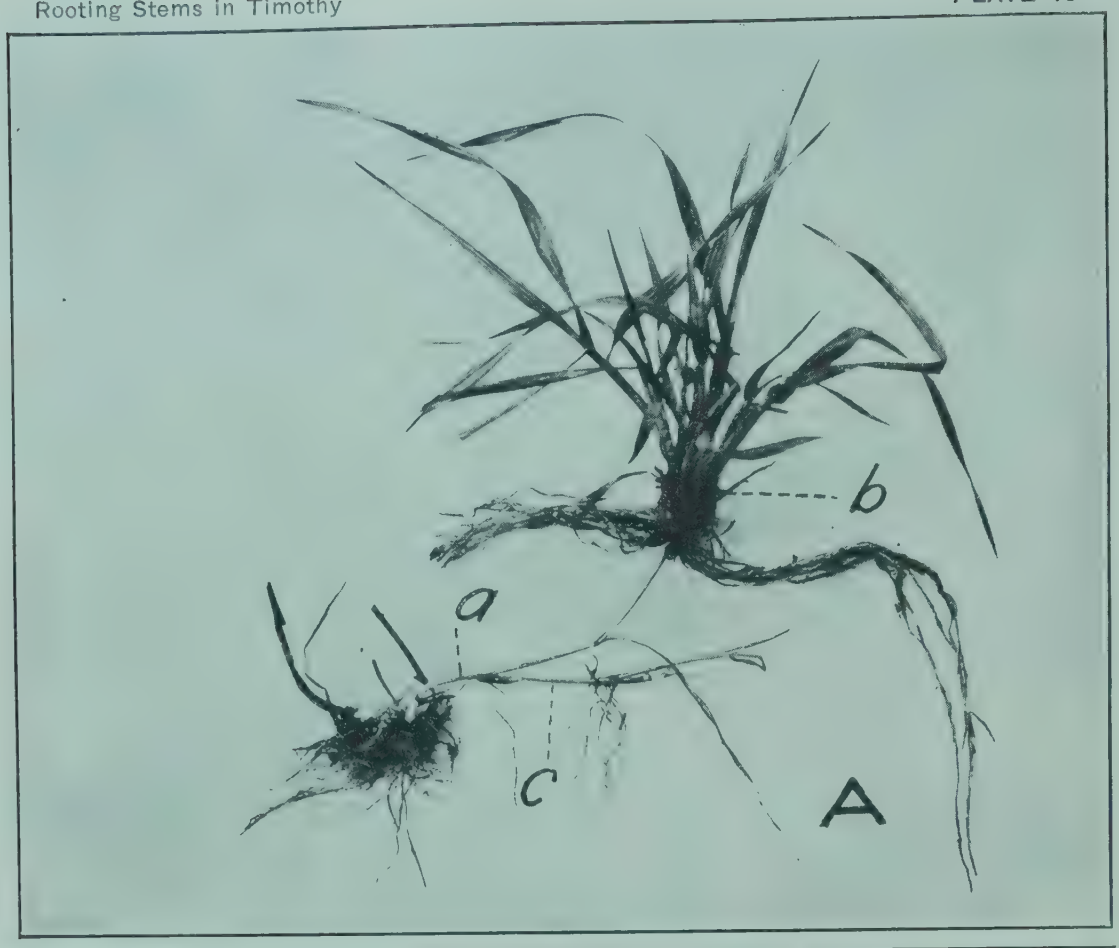
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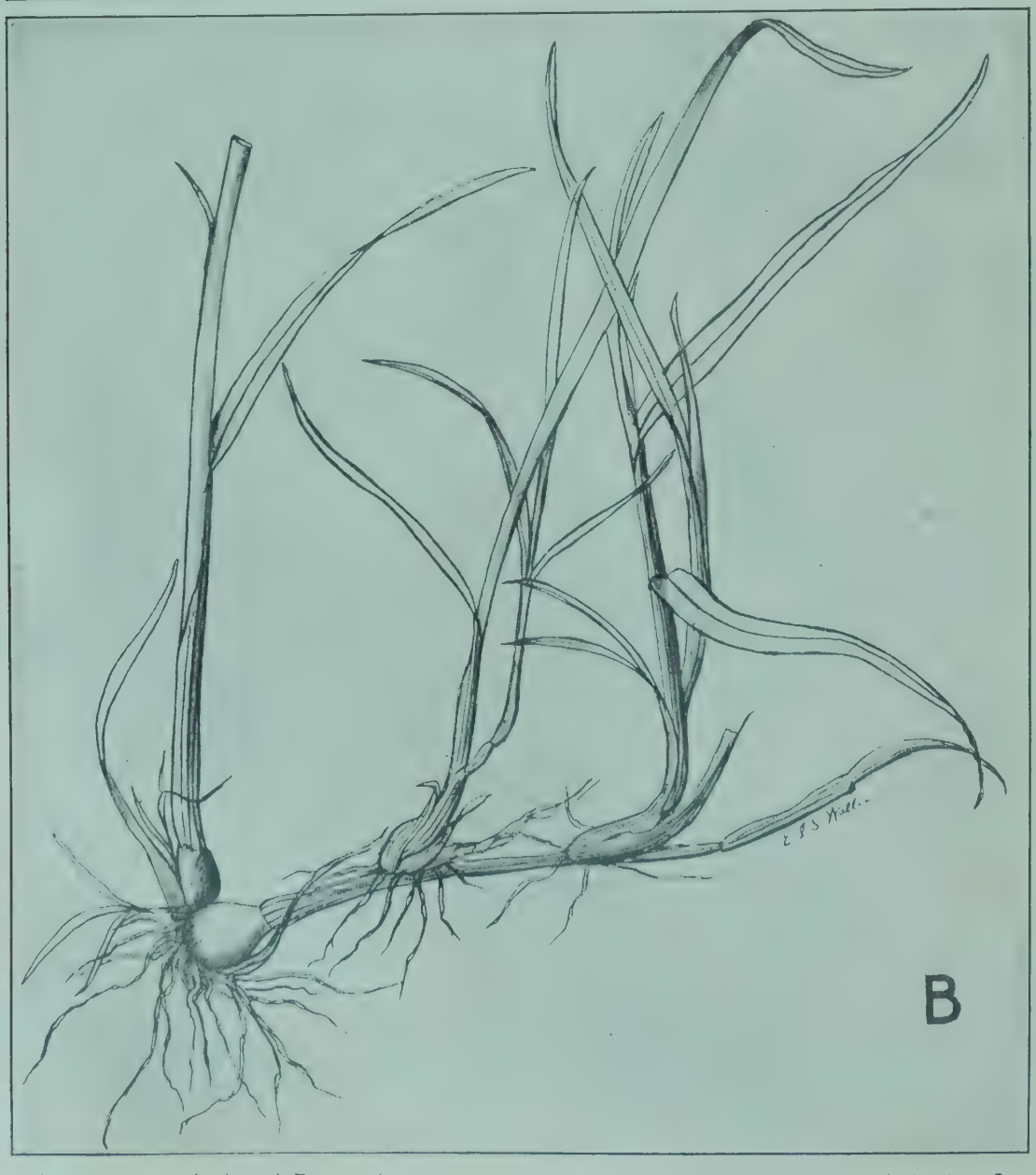
B

PLATE 40

A.—Timothy plants arising from a true culm that was plowed under. Note the roots at the node *a*.

B.—A decumbent culm of timothy functioning as a stolen (after Witte).

COLLAR-ROT OF TOMATO

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INTRODUCTION

A new disease of tomato seedlings, which takes the form of a rotting and girdling of the stems at the surface of the soil, has for the past three years caused heavy loss in Maryland, New Jersey, and Delaware.

This is chiefly a seed-bed disease. Young, tender plants infected in the seed bed carry the disease to the field. Although field infections may occur also they do not appear to be common. Moreover, the hardened condition of the plants and the relative freedom of the field from the causal parasites make the opportunities for infection less favorable than in the seed bed.

The most prominent symptom of the disease is the dark brown lesions on the stem at the surface of the ground (Pl. 41, A, *a*, *c*) which often encircle the stems, forming a collar. These lesions also occur on other parts of the stem (Pl. 41, A, *b*) but not so frequently as at the soil line. They enlarge and make the affected parts weak and brittle. There is very little infection of the roots.

Diseased plants set in the field are commonly snapped off by the wind at the point of infection. Some recover by forming a callus over the wound, and others outgrow it by forming new roots above the diseased area. Plants which recover are seldom as productive, however, as those which have always been healthy. The estimated loss of the crop from this disease in Delaware in 1919 was 30 per cent.

Rosenbaum [1] attributes the disease to *Macrosporium solani*, and Cook [2] to Rhizoctonia. The writers have isolated from collar-rot material a Verticillium that also readily infects tomato seedlings and causes the typical symptoms of the disease.

The present paper contains the results of inoculation experiments made with this species of verticillium and also with *Macrosporium solani* and *Rhizoctonia solani*.

EXPERIMENTAL WORK

MATERIAL AND METHODS

The Verticillium used in these experiments was isolated from young tomato plants infected by collar-rot in seedbeds in Maryland. The

[1] ROSENBAUM, Joseph. A STEM DISEASE OF TOMATO CAUSED BY MACROSPORIUM SOLANI E. & M. (Abstract.) *In* Phytopathology, v. 10, no. 1, p. 59. 1920.

[2] COOK, Mel. T. THE ALTERNARIA FRUIT ROT AND RHIZOCTONIA STEM ROT OF TOMATOES. (Abstract.) *In* Phytopathology, v. 10, no 1, p 59. 1920.

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culture of *Macrosporium solani* was obtained from L. O. Kunkel and that of *Rhizoctonia solani* from M. Shapovalov. Three strains of *R. solani* were used in the inoculation of 400 plants, but as all three showed about the same degree of infectiousness only one was used in later experiments.

Most of the tomato plants used were 2 to 3 inches high, but some were 5 to 6 inches, and a few 10 to 12 inches in height. All were in good growing condition.

The inoculations were made by placing mycelium from a fresh culture against the stem, at or slightly below the surface of the soil. From cultures of Verticillium both mycelium and spores were used. Usually the epidermis was not injured before applying the inoculum, but in a few experiments it was lightly scratched. To keep the fungus moist until it had time to infect the stems, the soil was thoroughly watered before the plants were inoculated. By placing the fungus between the stem and this moist soil it was kept wet. Usually the plants were left standing in the greenhouse after inoculation, but in a few experiments they were kept in a moist chamber 48 hours.

Repeated inoculation experiments were made with Verticillium, several hundred plants being inoculated and as many more used as controls.

RESULTS OF INOCULATION WITH VERTICILLIUM

Young tender seedlings, such as grow in a seed bed, were very easily infected by this fungus (Pl. 42, A). Plants that had grown slowly and were somewhat woody were less susceptible and not infrequently recovered from the disease. Larger plants 10 to 12 inches high were scarcely susceptible. Infection was obtained on more than 80 per cent of the inoculated plants 1 to 6 inches high, while only an occasional infection, 1 or 2 per cent, occurred on the control plants. The latter was due to the presence of a small amount of the fungus in the greenhouse soil.

Nearly all these infections were obtained without previously injuring the epidermis. In fact, puncturing the epidermis seemed to make little difference, as the fungus was able to penetrate the tissues very easily in either case.

DESCRIPTION OF FUNGUS

Verticillium lycopersici, n. sp.

Dense colonies (Pl. 41, B), at first white and later bluish green, are formed by this fungus on most culture media. The conidiophores are distinctly septate at the base (Pl. 43, D), 70 to 350 μ (occasionally 700 μ or more) in length, verticillately branched. The primary whorls average 5 to 7 in number; the secondary whorls of each branch average 1 to 3. The sterigmata are hyaline, somewhat bottle-shaped (Pl. 43, B, C), and 7.5 to 11.5 μ by 3.5 to 4 μ in size. The conidia (Pl. 43, C, E, F), are globoid to ellipsoid, the globoid being 3.8 to 4.7 μ in diameter, the ellipsoid 3.8 to 4.7 μ by 4 to 5.8 μ in transverse and longitudinal dimensions. They are bluish green on most culture media but yellowish on potato agar. From tips of the sterigmata they are cut off singly but frequently adhere in heads of the so-called Acrostalagmus type.

COMPARATIVE INFECTIOUSNESS OF VERTICILLIUM LYCOPERSICI, MACROSPORIUM SOLANI, AND RHIZOCTONIA SOLANI

Experiments were made to determine the relative infectiousness of these fungi and the difference in type of lesion they produce. Small plants were inoculated as previously described by placing mycelium or mycelium and spores from a fresh culture against the stem at or a little below the soil line.

Table I gives the number and percentage of infections obtained within two to three weeks after the inoculations were made.

TABLE I.—*Results of inoculating tomato seedlings with collar-rot fungi when plants were not placed in a moist chamber*

Fungus.	Epidermis lightly scratched before inoculation.			Epidermis not injured before inoculation.		
	Number of plants inoculated.	Number of plants infected.	Percentage of plants infected.	Number of plants inoculated.	Number of plants infected.	Percentage of plants infected.
Verticillium lycopersici	180	180	100	466	372	80
Macrosporium solani	57	57	100	380	319	84
Rhizoctonia solani	92	47	51	466	72	15
Control	.....	.....	.....	[a] 86	1	1

[a] Number of plants used but not inoculated.

As shown by the table, *Verticillium lycopersici* and *Macrosporium solani* produced a high percentage of infections even when applied to an uninjured epidermis. *Rhizoctonia solani*, on the other hand, produced a much smaller percentage of infections, especially when the epidermis was not injured in the process of inoculation.

In order to determine whether *Rhizoctonia solani* would have produced a higher percentage of infections under more humid conditions another set of inoculation experiments was made in which the plants were kept in a moist chamber 48 hours after inoculation. The results are shown in Table II.

TABLE II.—*Results of inoculating tomato seedlings with collar-rot fungi when plants were kept in a moist chamber 48 hours after inoculation*

Fungus.	Epidermis lightly scratched before inoculation.			Epidermis not injured before inoculation.		
	Number of plants inoculated.	Number of plants infected.	Percentage of plants infected.	Number of plants inoculated.	Number of plants infected.	Percentage of plants infected.
Verticillium lycopersici	6	6	100	21	19	90
Macrosporium solani	.....	.....	.....	27	23	85
Rhizoctonia solani	18	18	100	33	4	12
Control	.....	.....	.....	[a] 15	2	13

[a] Number of plants used but not inoculated.

The percentage of infections by *Rhizoctonia solani* was higher on the scratched series than in the previous experiments but no higher in the unscratched series. *R. solani* seems to require high humidity and a punctured epidermis to cause much infection and is, therefore, a weaker and more limited parasite on tomato than either *Verticillium lycopersici* or *Macrosporium solani*. Moreover, the infections caused by it were more superficial and usually disappeared as the plants became older.

The infected areas produced by *Macrosporium solani* were usually a little darker in color than those made by *Verticillium lycopersici* or *Rhizoctonia solani*, especially on the older seedlings.

In order to determine whether the relative infectiousness of these fungi is the same in soil as when applied to the stems, sterilized soil was mixed with each culture, and in this were planted tomato seeds that had been treated with a solution of bichlorid of mercury 1 to 1,000 to destroy adhering parasites and had been washed in distilled water. The results obtained three weeks after this experiment was started are summarized in Table III.

TABLE III.—*Comparative infectiousness of collar-rot fungi in tomato seed bed*

Fungus.	Number of plants grown.	Number of plants affected by collar-rot.	Percentage of plants affected by collar-rot.
Verticillium lycopersici	567	362	64
Macrosporium solani	420	307	73
Rhizoctonia solani	755	20	3
Control	282	0	0

The results show that *Verticillium lycopersici* and *Macrosporium solani* produced nearly as high percentages of collar-rot infections as in the previous experiments in which the mycelium was placed directly in contact with the stem. Moreover, there was no distinguishable difference in the color or other appearance of the lesions produced by these two fungi. On the contrary, *Rhizoctonia solani* produced very few infections. In fact, under the conditions of this experiment it was not a very active parasite.

The minimum growth temperatures obtained with cultures of these fungi were 1.5° C. for *Macrosporium solani*, 12° for *Verticillium lycopersici*, and 15° for *Rhizoctonia solani*. In cold seed beds *Macrosporium solani* would, therefore, begin to grow before either of the other fungi but would have little advantage over them, as tomato seed will not usually germinate until it reaches a temperature of 14° or 15°. Moreover, *Macrosporium solani* and *Rhizoctonia solani* grow and spread slowly while *Verticillium lycopersici* grows rapidly and spreads readily by means of innumerable spores. In seed bed or field it would therefore probably spread much faster than either of the other fungi.

CROSS INOCULATION

As the potato, which is sometimes grown in rotation with the tomato, and the horse nettle *Solanum carolinense* L., which is a common weed in the Middle Atlantic States, are closely related to the tomato, inoculations of these plants were made with collar-rot fungi. Young shoots were inoculated at the base of the stem with mycelium from a fresh culture, as described in previous experiments. The results are given in Table IV.

TABLE IV.—*Effect of inoculating potato and horse nettle with collar-rot fungi*

Fungus.	Host.	Number of shoots inoculated.	Number of shoots infected.	Percentage of shoots infected.
Macrosporium solani	Potato	43	38	88
Verticillium lycopersici	do	47	25	53
Rhizoctonia solani	do	41	1	2
Control	do	45	0	0
Macrosporium solani	Horse nettle	50	48	96
Verticillium lycopersici	do	50	34	68
Rhizoctonia solani	do	50	5	10
Control	do	50	0	0

The percentage of infections produced on potatoes was higher for *Macrosporium solani* than for *Verticillium lycopersici*. Moreover, the lesions caused by *M. solani* were very prominent (Pl. 42, B), while those caused by *V. lycopersici* (Pl. 44, A) were smaller and more superficial. Only one potato shoot inoculated with *Rhizoctonia solani* was infected. As shown by Edson and Shapovalov,[1] strains of *R. solani* differ markedly in their power of infecting potato plants. Although we used on potato the strain that seemed to give the best results on tomato, it was evidently not a strong potato parasite.

Since all the potato control plants (Pl. 44, B) were healthy, the comparative results obtained with these three fungi are probably reliable.

The results on horse nettle are also of importance, since they show that all three parasites are able to infect this plant. *Macrosporium solani* and *Verticillium lycopersici* (Pl. 45) produced numerous, well-marked lesions. *Rhizoctonia solani* produced only a few, but they suffice to show that it can infect this host.

As the source of this disease is at present chiefly in the seed bed, it can be largely controlled by sterilizing the seed-bed soil. If allowed to go unchecked it may ultimately accumulate in fields in which conditions are favorable for its development. Since its spread will be facilitated by susceptible hosts, both the potato and horse nettle should be kept out of prospective tomato fields in collar-rot infested areas.

[1] EDSON, H. A., and SHAPOVALOV, M. POTATO-STEM LESIONS. *In* Jour. Agr. Research, v. 14, no. 5, p. 213–220, pl. 24–26. 1918.

SUMMARY

Collar-rot of tomatoes has been prevalent in Maryland, New Jersey, and Delaware during the three seasons 1917, 1918, and 1919, destroying many seedlings in the seed bed and newly set plants in the fields.

The girdling of the stem at the surface of the ground characteristic of the disease may be produced by three fungi, *Verticillium lycopersici*, *Macrosporium solani*, and *Rhizoctonia solani*.

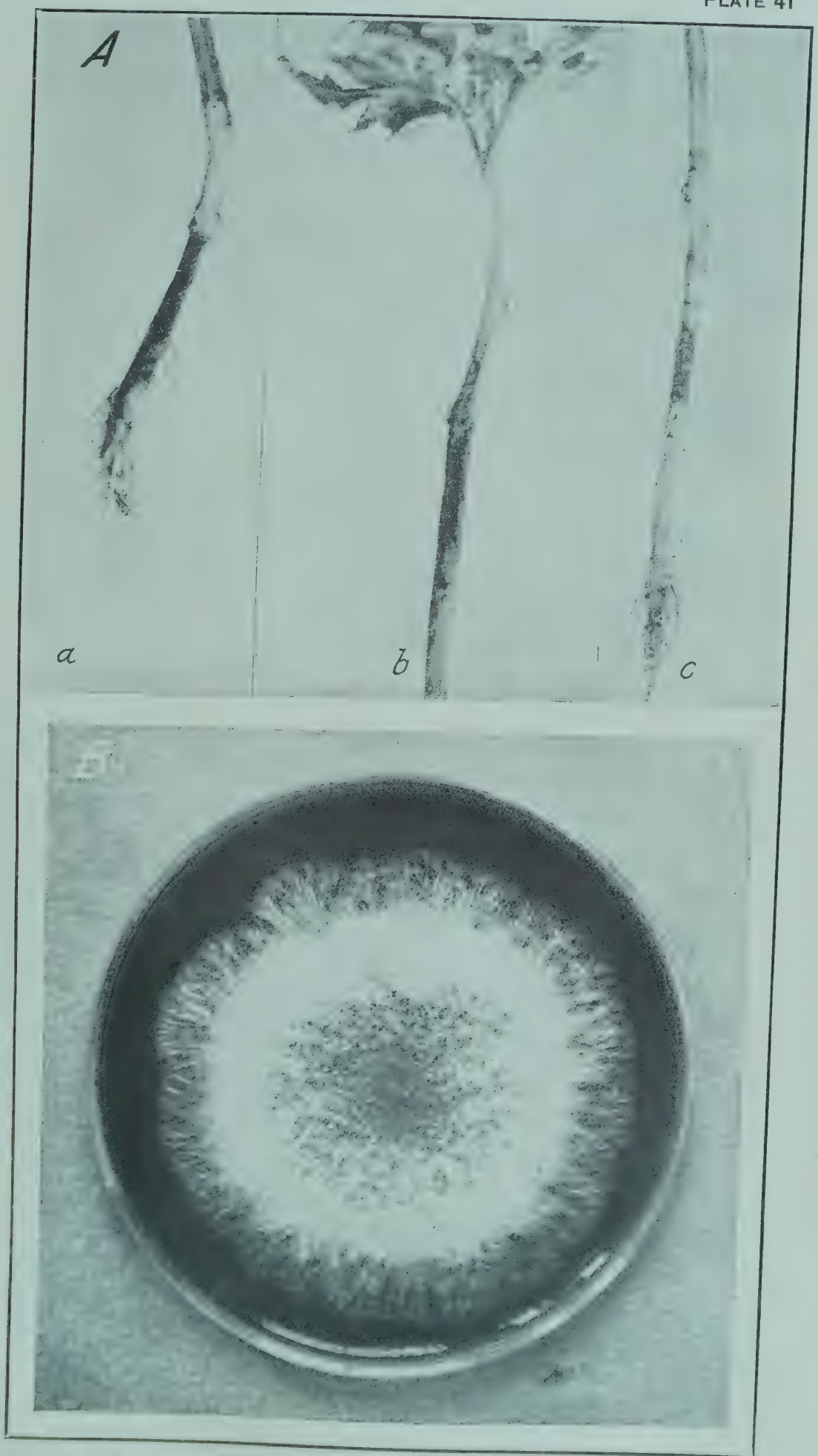
In inoculation experiments with these fungi, made either by applying fresh cultures to uninjured stems or by mixing them with the potted soil, *Verticillium lycopersici* and *Macrosporium solani* infected tomato seedlings about equally well, causing typical lesions on 65 to 100 per cent of the plants used, while Rhizoctonia produced a very few infections of a superficial nature.

On stems of potato and horse nettle all three fungi produced typical collar-rot lesions, but the percentage of infections was much higher for *Verticillium lycopersici* and *Macrosporium solani* than for *Rhizoctonia solani*.

PLATE 41

A.—Tomato seedlings several weeks old affected by collar-rot. Natural infection. *a*, *c*, Infection near surface of soil; *b*, infection near tip of stem.

B.—Typical young colony of *Verticillium lycopersici* on bean agar.


A
a
b
c

A

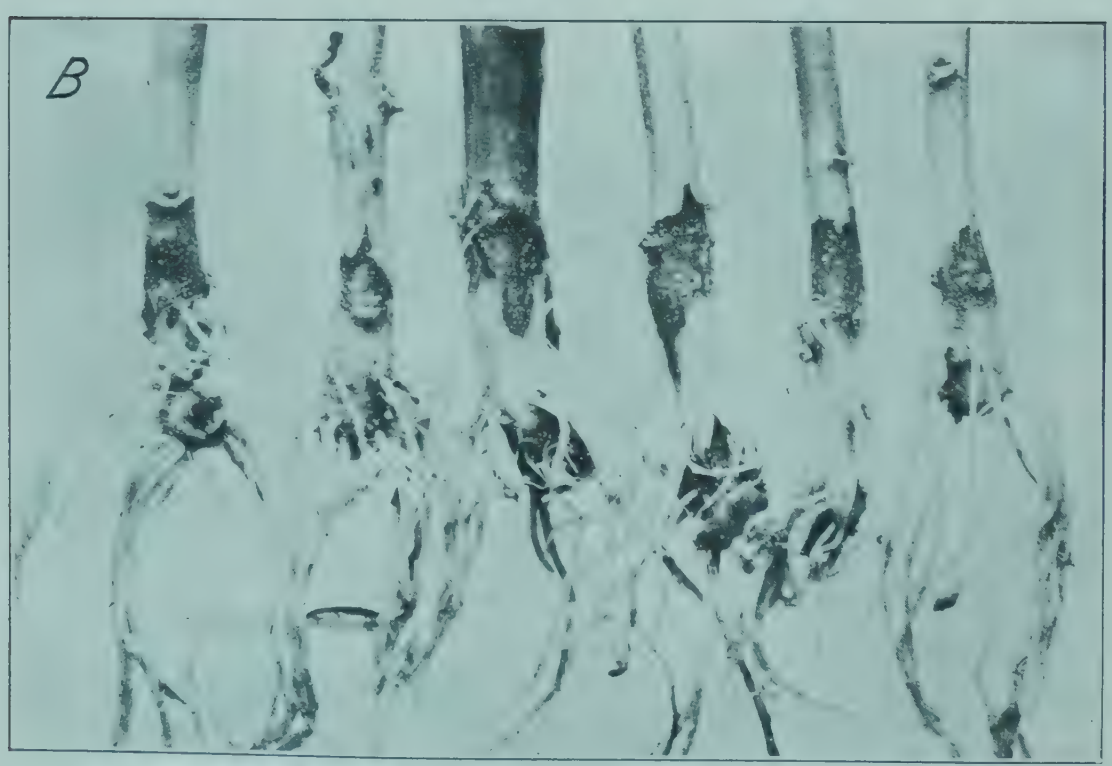

B

PLATE 42

A.—Young tomato seedlings 16 days after inoculation with *Verticillium lycopersici*. Epidermis not injured by process of inoculation.

B.—Potato stems infected by *Macrosporium solani*. Inoculated with pure culture without injuring epidermis. Photographed 23 days after inoculation.

PLATE 43

Stages in development of *Verticillium lycopersici:*

A.—Outer end of conidiophore, showing characteristic branching and aggregation of conidia.

B.—End of conidiophore more highly magnified than in A.

C.—Sterigmata with conidia forming singly at the tips and adhering in heads.

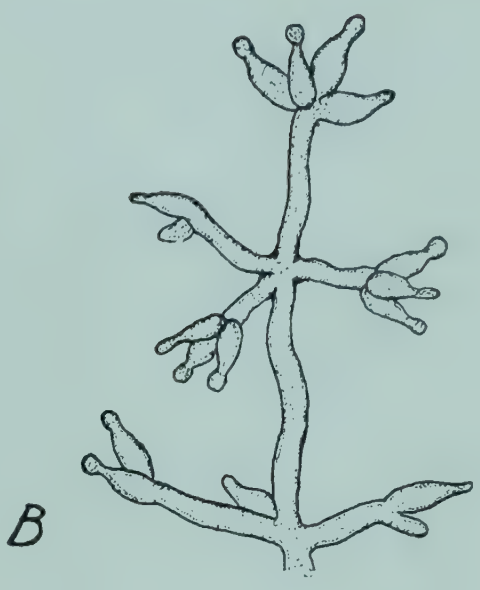
D.—Characteristic septation in lower whorls of conidiophores.

E.—Conidia as seen in air or in water mount. In plate cultures they are found in both separated and aggregated conditions.

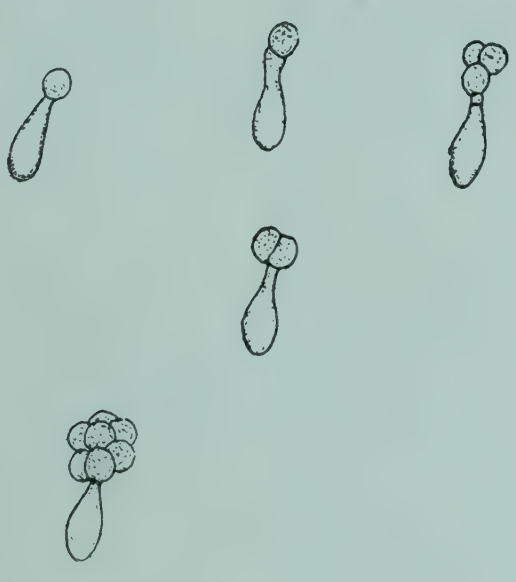
F.—Germinating conidia.

All drawings except A were made on a level with the table with camera lucida and Zeiss apochromatic 4-mm. objective and compensating ocular 6 and were reduced one-third.

A

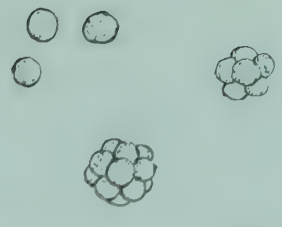


B

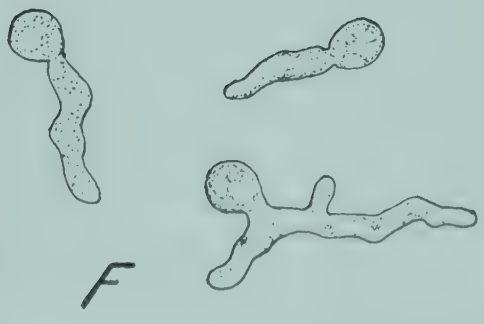


C

D



E



F

A

B

PLATE 44

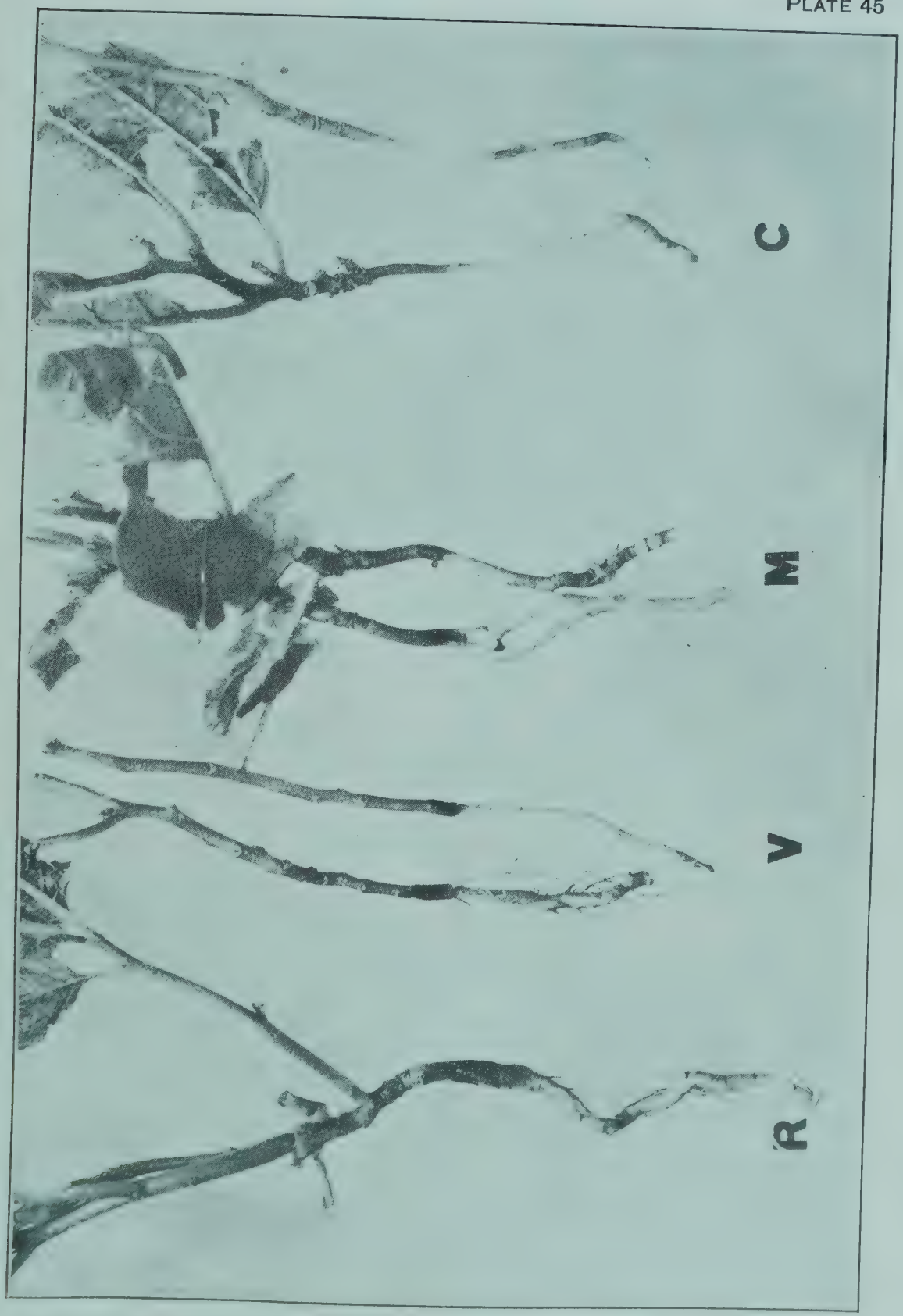
A.—Potato stems infected by *Verticillium lycopersici*. Inoculated with pure culture without injuring epidermis. Photographed 23 days after inoculation.

B.—Healthy potato stems used as controls with inoculated stems shown in Plates 42, B, and 44, A. Photographed 23 days after beginning of experiment.

PLATE 45

Horse nettle (*Solanum carolinense*) stems infected by the following fungi: R, *Rhizoctonia solani;* V, *Verticillium lycopersici;* M, *Macrosporium solani;* C, not inoculated (control plants).

Inoculated with pure cultures without injuring epidermis. Photographed 19 days after inoculation.


C
M
V
R

BACTERIAL LEAFSPOT DISEASE OF CELERY

By Ivan C. Jagger

Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture

Since 1910 an undescribed bacterial leafspot disease of celery has been under observation. A short paper [1] was read at the 1914 meeting of the American Phytopathological Society. The disease has been widely distributed in western and central New York State each season, occurring on all varieties of celery commonly grown, including various strains of Golden Self-Blanching and numerous varieties and strains of "green" celery. Dr. C. H. Coons [2] states that it has been of common occurrence in Michigan during the past few seasons.

The spots are of a rusty brown color, irregularly circular in outline, and rarely exceed 5 mm. in diameter (Pl. 46, 47). They closely resemble the Septoria leafblight spots and can be distinguished with certainty only by the absence of pycnidia, which show as black dots in the Septoria spots. Occasionally the spots are so numerous as to cause the death of many of the older leaves, but usually the injury consists in the disfiguring of the foliage and in a possible reduction in growth of the plants. The disease seems to be confined to the leaf blades, spots seldom, if ever, occurring on the petioles.

Bacteria have been repeatedly isolated and the characteristic spots reproduced by inoculation with pure cultures. In the greenhouse during the winter season the organism, sprayed on uninjured leaves, has repeatedly failed to give infection although inoculations through needle punctures have invariably resulted in characteristic leafspots. Similar inoculations by spraying the organism from pure cultures on uninjured leaves have resulted in abundant infection during the summer season under field conditions.

The causal organism is a short rod, measuring when stained from 3-day agar slants 0.44 to 0.87 μ by 0.87 to 1.74 μ. It has one to several, usually one to three, polar flagella.

In the study of the cultural features the methods given by Smith[3] were followed as far as possible. All observations were made on cultures held at 20° C. Growth on standard nutrient agar is rather rapid, colonies being evident in dilution plates in 48 hours or less; at the end of 3 days surface colonies are 2 to 5 mm. in diameter, grayish white, translucent,

[1] Jagger, Ivan C. A bacterial leaf spot disease of celery. (Abstract.) *In* Phytopathology, v. 4, no. 6, p. 395. 1914.

[2] Coons, G. H., and Nelson, Ray. The plant diseases of importance in the transportation of fruits and vegetables. Circ. 473-A, Amer. Railway Perishable Freight Assoc., p. 32, fig. 51. 1918.

[3] Smith, Edwin F. Bacteria in relation to plant diseases. 3 vol. Washington, D. C. 1905-1914.

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May 2, 1921
Key No. G-228.

shiny, circular, edges entire, flat to slightly raised; buried colonies are 0.2 to 0.5 mm. in diameter, spherical to lens-shaped, white opaque. Growth on agar streaks is similar, cultures several days old usually showing a granular structure and greenish discoloration of the medium. Agar stabs show only slight clouding along the puncture with abundant surface growth. Gelatin is liquefied, usually rapidly, various strains of the organsim, however, showing marked variation in rate of liquefaction. In nutrient broth there is strong turbid clouding, no surface growth, and more or less ropy viscid sediment in 10-day and older cultures. Litmus milk becomes more alkaline, and the casein is usually peptonized without the formation of curd. Fermentation tubes containing tap water, to which has been added 2 per cent peptone and 0.5 per cent sodium chlorid plus 1 per cent, respectively, of lactose, dextrose, saccharose, and glycerin, show no growth in the closed arm and no gas formation. Growth in the open arm is abundant, and litmus paper shows marked production of acid from glucose, less marked production of acid from saccharose, and alkali production from lactose and glycerin. Nitrates are not reduced. There is no growth in Cohn's and Uschinsky's solutions. Growth on cooked potato plugs is abundant and grayish to yellowish white with no indications of diastatic action on the starch.

The group number of the organism is 211.2322033, following the third descriptive card of the Society of American Bacteriologists,[1] adopted in 1907. In the classification recently proposed by the society [2] the organism belongs to the genus Pseudomonas, which is also in accord with the system of Migula. In Smith's [3] system it falls in the genus Bacterium.

Pseudomonas apii, n. sp.

A short rod, 0.44 to 0.87μ by 0.87 to 1.74μ, one to several polar flagella; colonies on nutrient agar grayish white, shiny, circular, edges entire, flat to slightly raised, granular with age; gelatin liquefied, litmus milk becomes more alkaline, casein peptonized without formation of curd; acid formed from glucose and saccharose, alkali from lactose and glycerin, no gas; no growth in closed arm of fermentation tubes; nitrates not reduced; no diastatic action; no growth in Cohn's and Uschinsky's solutions. Group number 211.2322033.

Parasitic in leaves of celery (*Apium graveolens* L.); distribution, New York, Michigan.

Potebnia [4] has described a leafspot of cultivated parsley in Russia caused by *Bacillus petroselina*. The close relationship of parsley and

[1] Society of American Bacteriologists. descriptive chart. Prepared by committee on methods of identification of bacterial species. Endorsed by the Society for general use at the annual meeting, Dec 31, 1907.

[2] Winslow, C., E. A., et al. the families and genera of the bacteria. Final report of the committee of the Society of American bacteriologists on characterization and classification of bacterial types. *In* Jour. Bact., v. 5, no. 3, p. 191–229. 1920. References, p. 226–229.

[3] Smith, Erwin F. op. cit.

[4] Potebnia, A. A. gribnye parasity vysshikh rastenii kharkovsk. i smerzhnykh gubern. (fungous parasites of the higher plants in kharkov and adjacent provinces. Kharkov Obl. Selskokh. Opyt. Sta., No. 1, List. 1/8, p. 1–120, illus. 1915. Review by Michael Shapovalov *in* Phytopathology, v. 6, no. 3, p. 293–295. 1916.

celery suggests the possibility that the celery disease is caused by the same organism. A limited number of inoculations with the celery organism have failed to give infection on parsley. Potebnia states that his organism does not liquefy gelatin, whereas the celery organism invariably causes liquefaction.

During the seasons 1915–1917 spraying, as a means of controlling the disease, was tested at Irondequoit, N. Y. All applications were made with a hand sprayer, maintaining a pressure of 25 to 50 pounds. Care was taken to cover the leaves thoroughly with the spray material. Several rows were sprayed with standard 5–5–50 Bordeaux mixture each season. During the last two seasons comparable rows were sprayed with commercial lime-sulphur solution, testing 32° Beaumé, which was diluted 1 part to 25 parts of water. The first application was made soon after the plants were transplanted from the seed bed to the field and was repeated at intervals of one to two weeks until a few weeks before harvest. In 1915, applications were made on August 9, 21, September 1, 10 and 22; in 1916, on August 12, 19, 28, September 6, 16, 26, and October 6; and in 1917 there were a similar number of applications.

Each season data were taken in October, a few days before harvest. The number of bacterial leafspots were counted on representative plants occupying opposite positions in adjacent sprayed and unsprayed rows. A part of the data is given in Table I. Sets of figures separated by horizontal spaces are for opposite and comparable plants. The number of spots on each leaf is given separately, the leaves being numbered consecutively from the oldest to the youngest fully expanded leaf.

In 1917 Dr. H. W. Dye of the Cornell Agricultural College made three applications of standard 5–5–50 Bordeaux mixture at intervals of about nine days at Williamson, N. Y., to celery which already showed the bacterial leafspot disease. A few days after the last application the number of bacterial leaf spots was counted on 10 sprayed plants and 35 comparable unsprayed plants. The average number of spots per plant was for the sprayed 214 and for the unsprayed 997.

The data presented seem to show conclusively that the disease is very satisfactorily controlled by spraying with Bordeaux mixture but is not controlled with lime-sulphur solution. General appearances of the sprayed and unsprayed rows in the field led in every case to the same conclusion.

TABLE I.—*Number of bacterial leafspots on the leaves of sprayed and unsprayed celery plants*

Treatment (spray material applied).	Number of spots on individual leaves, numbered from oldest to youngest—												Total number leaf spots on plant.	Year.
	1	2	3	4	5	6	7	8	9	10	11	12		
Control	98	94	74	62	63	51	10	0	0	0	....	....	452	1915
Bordeaux	2	1	3	0	0	0	0	0	0	0	....	....	6	
Control	195	77	17	16	42	0	0	0	0	0	....	....	347	
Control	425	82	144	60	36	0	0	0	0	0	....	....	747	1915
Bordeaux	64	18	1	5	0	0	0	0	0	0	....	....	88	
Control	475	250	73	93	32	38	0	0	0	0	0	....	961	
Control	147	117	48	32	25	13	4	0	0	0	....	....	386	1915
Bordeaux	6	5	0	0	0	0	0	0	0	0	0	....	11	
Control	233	143	109	30	64	0	0	0	....	....	....	....	579	
Control	68	65	41	23	46	16	4	0	0	0	0	0	263	1916
Lime-sulphur	56	54	56	38	18	11	13	0	0	0	0	....	246	
Control	58	62	32	35	13	2	0	0	0	0	0	....	202	
Bordeaux	0	0	0	0	0	0	0	0	0	0	0	....	0	
Control	83	13	27	64	12	6	15	0	0	0	0	....	220	
Lime-sulphur	33	70	72	82	77	39	32	3	0	0	0	0	408	1916
Control	29	21	44	81	66	42	15	16	0	0	0	0	314	
Bordeaux	1	5	0	0	0	0	0	0	0	0	0	....	6	
Lime-sulphur	90	96	51	48	15	20	56	4	4	0	0	0	384	1917
Control	86	100	145	95	150	96	66	29	16	10	0	0	793	
Bordeaux	1	0	0	0	2	0	0	0	0	0	0	0	3	

SUMMARY

Pseudomonas apii, n. sp., is the cause of a leafspot disease of celery in New York and Michigan. The disease is controlled by spraying with Bordeaux mixture.

TABLE I.—*Number of bacterial leafspots on the leaves of sprayed and unsprayed celery plants*

Treatment (spray material applied).	Number of spots on individual leaves, numbered from oldest to youngest—												Total number leaf spots on plant.	Year.
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Bordeaux	2	1	3	0	0	0	0	0	0	0	....	....	6	
Control	195	77	17	16	42	0	0	0	0	0	....	....	347	
Control	425	82	144	60	36	0	0	0	0	0	....	....	747	1915
Bordeaux	64	18	1	5	0	0	0	0	0	0	....	....	88	
Control	475	250	73	93	32	38	0	0	0	0	0	....	961	
Control	147	117	48	32	25	13	4	0	0	0	....	....	386	1915
Bordeaux	6	5	0	0	0	0	0	0	0	0	0	....	11	
Control	233	143	109	30	64	0	0	0	....	....	....	....	579	
Control	68	65	41	23	46	16	4	0	0	0	0	0	263	1916
Lime-sulphur	56	54	56	38	18	11	13	0	0	0	0	....	246	
Control	58	62	32	35	13	2	0	0	0	0	0	....	202	
Bordeaux	0	0	0	0	0	0	0	0	0	0	0	....	0	
Control	83	13	27	64	12	6	15	0	0	0	0	....	220	
Lime-sulphur	33	70	72	82	77	39	32	3	0	0	0	0	408	1916
Control	29	21	44	81	66	42	15	16	0	0	0	0	314	
Bordeaux	1	5	0	0	0	0	0	0	0	0	0	....	6	
Lime-sulphur	90	96	51	48	15	20	56	4	4	0	0	0	384	1917
Control	86	100	145	95	150	96	66	29	16	10	0	0	793	
Bordeaux	1	0	0	0	2	0	0	0	0	0	0	0	3	

SUMMARY

Pseudomonas apii, n. sp., is the cause of a leafspot disease of celery in New York and Michigan. The disease is controlled by spraying with Bordeaux mixture.

PLATE 46

Bacterial leafspots on leaf of Golden Self-Blanching variety of celery.

Bacterial Leafspot Disease of Celery

PLATE 46

PLATE 47

Bacterial leafspots on leaflet of Golden Self-Blanching variety of celery. Approximately × 7/8.

36731°—21——7

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GLUCOSE AS A SOURCE OF CARBON FOR CERTAIN SWEET POTATO STORAGE-ROT FUNGI

By J. L. WEIMER, *Assistant Pathologist*, and L. L. HARTER, *Pathologist, Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The fact that fungi do not have the power to manufacture their own carbohydrates has long been known, and it has been demonstrated many times that carbon in certain chemical combinations is more available than in others. Some microorganisms can utilize cane sugar, while others can use only the invert sugars, and still others thrive upon organic acids, weak alcohols, and other organic compounds. Both cane sugar and invert sugars are commonly present in the sweet potato, and hence it is these two forms of carbon which should receive attention in the study of this phase of this problem.

Butkewitsch (*3*)[1] showed that *Rhizopus nigricans* Ehrb. could not utilize cane sugar as such, and, further, that this organism produced no invertase. On the contrary, Pringsheim and Zemplen (*26*) found that although *R. tonkinensis* Vuill., *Mucor javanicus* Wehm., *Penicillium purpurogenum* Fleroff., *P. africanum* Doeb., and *P. brevicaule* Sacc., could not use cane sugar directly, they did possess the power to invert it. Ritter (*28*) worked with several of the same forms and demonstrated that some of the organisms were able to invert cane sugar while others were not. He showed further that when ammonium nitrate or ammonium sulphate were employed as a source of nitrogen, nitric acid or sulphuric acid was formed, which probably inverted some of the cane sugar, thus making it available as a source of carbon for the fungi.

The investigations of Hasselbring and Hawkins (*15*) showed that the cane sugar, and to some extent the glucose content of the sweet potato in storage, increased with a decrease in starch from the time of digging in October until March or April, when there was a reverse of the process.

They pointed out, also, that the amount of glucose in the sweet potato is always small as compared with that of the cane sugar. It is believed by many that the susceptibility of the sweet potato to decay likewise increases as the season progresses. This might seem to imply that there is some correlation between the sugar content of the potato and

[1] Reference is made by number (italic) to "Literature cited," p. 208–210.

its susceptibility to disease. At the time these investigations were initiated by the writers there were little available data regarding the sugar requirements of the particular fungi responsible for the decay of sweet potatoes in storage. Since glucose is probably the most readily available carbohydrate in the sweet potato for a majority of the fungi to be studied, experiments were designed to determine to what extent this sugar is utilized as a source of carbon by the following organisms: *Fusarium acuminatum* E. and E. emend. Wollenw., *Diplodia tubericola* (E. and E.) Taub., *Rhizopus tritici* Saito, *Mucor racemosus* Fes., *Sclerotium bataticola* Taub., *Penicillium* sp., *Botrytis cinerea* Pers., and *Sphaeronema fimbriatum* (E. and H.) Sacc.

EXPERIMENTAL METHODS

CULTURE MEDIA

Many different synthetic culture media have been used by investigators while studying the physiology of fungi. When the present investigations were begun, Czapek's nutrient solution, together with several others, were tested, but none proved entirely satisfactory. In trial experiments with *Rhizopus tritici* it was found that when ammonium nitrate was substituted for sodium nitrate and glucose for cane sugar in Czapek's solution a vigorous growth resulted. Other storage-rot fungi responded similarly. Hence, 1.6 gm. of ammonium nitrate were used instead of the 2 gm. of sodium nitrate required per 1,000 cc. in Czapek's solution. The solution as finally employed was prepared as follows:

Magnesium sulphate ($MgSO_4$)	0.5 gm.
Potassium phosphate (K_2HPO_4)	1.0 gm.
Potassium chlorid (KCl)	.5 gm.
Ferric sulphate ($FeSO_4$)	.01 gm.
Ammonium nitrate (NH_4NO_3)	1.6 gm.
Distilled water	1,000 cc.

Only C. P chemicals were used in preparing this solution.

That fungi differ in their requirements as to the source of nitrogen, as well as carbon, has been suggested by the results of many investigators. Laurent (*20*) found that *Alternaria tenuis* Nees, *Mucor racemosus* Fres, and *Aspergillus glaucus* Link, grew well on solutions containing nitrates. *Aspergillus niger* v. Tieg., on the other hand, although it can utilize nitrates as a source of nitrogen, thrives better on ammonia. It is claimed by some investigators that a combination of peptone and glucose gives the best results with most fungi. Went (*31*) found that this combination was preferable to all others for Monilia. Czapek (*5*), on the other hand, showed that the amino-acids in association with glucose are preferable to peptone for Aspergillus, and Fischer (*8*) found that *Bacillus coli* Esch, *B. subtilis* (Ehren) Cohn, and *B. pyocyanus* Gess, could use nitrate in association with glucose, but if glycerin was substituted for the glucose the latter organism alone thrived. Dox (*6*) found that species of Asper-

gillus and Penicillium grew well on Czapek's nutrient solution, which contains its nitrogen and carbon in the form of sodium nitrate and cane sugar, respectively. Young (*32*) on the other hand, used ammonium nitrate as a source of nitrogen and cane sugar as a source of carbon for *Aspergillus niger* to good advantage.

The results of various workers seem to indicate that no generalizations can be made as to the best source of nitrogen and carbon for fungi. They rather point to the fact that the various fungi differ in their requirements and that the best source of both these elements must be ascertained for each organism.

It is not claimed that the culture medium used for these investigations is the best for all the fungi studied. It is likely that a better medium might be found for each of the organisms. The nature of the work, however, required that the same substrate be used for all the fungi, and the modification of Czapek's nutrient solution as here employed seemed to meet the requirements better than any other solution tried. Only one organism (*Sphaeronema fimbriatum*) failed to thrive well upon it.

PREPARATION OF CULTURES

A sufficient quantity of medium was prepared at one time for an entire experiment, thus insuring an equitable distribution of the salts. The solution was steamed for 20 minutes in an Arnold sterilizer and then filtered to remove a slight precipitate which formed during the heating. A definite quantity by weight of this solution was then placed into each of seven large beakers, and the required amount of Baker's C. P. dextrose was added to all but one to make approximately the following strengths of the carbohydrate: 00, 10, 20, 30, 40, 50, and 60 per cent. The solutions were then steamed in the beakers for 20 minutes and again filtered. One hundred fifty cc. of each solution were added to four 300-cc. Erlenmeyer flasks previously stoppered with cotton and weighed, thus making 7 sets of 4 flasks each containing an approximately equal amount of solution with six different strengths of dextrose and one without sugar. These flasks were reweighed and sterilized by steaming for 20 minutes on three consecutive days.

INOCULATION OF CULTURES

Three flasks of each set were inoculated with a loop of a heavy spore suspension in sterile distilled water or with mycelium from young and vigorous cultures growing either on Irish potato cylinders or stems of *Melilotus alba* Desr. The fourth flask in each set not inoculated was held as a control. The cultures were incubated in the dark for two weeks at about 28° C. Notes were taken on the general character of the growth at the end of the first week and again when the experiment was terminated.

METHOD OF TAKING RESULTS

At the end of two weeks the flasks with their contents were weighed again, and the mycelium was filtered out with the aid of suction into previously burned and tared alundum crucibles. The fungous felts were washed by pouring through the crucibles a quantity of distilled water and were then dried to constant weight in a vacuum oven at 60° C. The glucose content of all the solutions was determined by means of the Fric saccharimeter.

In determining the sugar content of the 10 and 20 per cent solutions, three and two times the normal weight of the solutions, respectively, were used, while the normal weight was taken in all the higher concentrations. The solutions were then made up to 100 cc. with distilled water and polarized through a 200-mm. tube.

The control solution produced no visible polarization.

The osmotic pressure and saccharimeter readings were made with freshly filtered solutions on the day the experiment was terminated. The depression of the freezing point was determined with a Beckman thermometer in an ordinary DeWar flask, the solution being cooled by the evaporation of ether. The osmotic pressure values were calculated from the freezing points obtained by one of the two formulas given by Harris and Gortner (*14*). When these were sufficiently low, as in series I, II, III, and IV, the first formula was used and the osmotic pressure was taken from their table, or from that given by Harris (*13*). This takes into consideration the error due to undercooling, whereas the second formula does not.

The changes in acidity of the solutions were determined by the use of a potentiometer, with the arrangement of Michaelis (*23*). The measurements were made in a closed vessel provided with a dip hydrogen electrode similar to that of Bovie (*1*), except that an exit tube was provided for the hydrogen. In most cases a day or two intervened before the potentiometer readings were made. In such cases the solutions were sterilized by steaming for 20 minutes and were then kept at a temperature of about 9° C. until used. A number of preliminary tests with these and other solutions showed that the hydrogen-ion concentration was not appreciably altered by steaming.

EXPERIMENTAL DATA

PERCENTAGE OF GLUCOSE REMAINING AT THE END OF THE EXPERIMENT

As already pointed out, the culture solutions were prepared to contain glucose in concentrations from 0 to 60 per cent. In the tables which follow, the controls are the flasks of the different series which were not inoculated.

An inspection of the control columns in Table I shows how nearly the solutions made roughly to contain the same percentages of sugar agree

in the corresponding series of different experiments. The 10 per cent solutions by weight showed a range of from 9.1 per cent to 9.7 per cent at the termination of the experiments, while those made up to contain 20 per cent tested from 18.2 per cent to 19.5 per cent, and so on, as indicated in the control columns in each series and under each organism.

TABLE I.—*Percentage of glucose remaining in the solutions at the end of the experiment* [a]

[Saccharimeter readings]

Series No.	Percentage of glucose by weight.	Solutions.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus tritici.*	*Mucor racemosus.*	*Sclerotium batalicola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control....	0	0	0	0	0	0	0	0
		Inoculated.	0	0	0	0	0	0	0	0
II	10	Control....	9.7	9.4	9.5	9.4	9.6	9.26	9.3	9.1
		Inoculated.	8.55	1.2	8.3	5.2	2.2	3.16	8.83	9.1
III	20	Control....	18.85	18.5	19.35	19.5	18.95	18.6	18.75	18.2
		Inoculated.	17.9	14.7	17.71	15.8	14.16	15.53	17.7	18.2
IV	30	Control....	28	26.8	29.2	28.7	27.8	28.1	28.5	25.5
		Inoculated.	27.1	23.3	27.2	26	25.4	23.46	26.03	25.5
V	40	Control....	37.1	38	39.7	38.1	37.3	37.5	37.8	37
		Inoculated.	36.7	37.3	36.8	36.9	36.7	32.86	37.8	.....
VI	50	Control....	46.1	48	49.2	47.8	45.5	46.9	47.7	.....
		Inoculated.	46.8	48.3	46.7	48.1	45.7	44.86	.....	.....
VII	60	Control....	57.5	57.3	56.4	58.8	57.1	57.9	.....	.....
		Inoculated.	.....	.....	.....	.....	.....	57	.....	.....

[a] Column 2 shows the percentage of glucose by weight, while the actual percentage of sugar present at the end of the experiments in each case is indicated by the saccharimeter readings given in the control columns. The significance of column 2 is the same in all the following tables.

For the sake of convenience in some of the general discussions reference will be made to the approximate percentages as shown in column 2 instead of to the actual percentage based on the saccharimeter readings.

The column headed "inoculated" represents the average of the concentrations of the three inoculated flasks of each series at the end of the experiment. For example, in series II of the *Fusarium acuminatum* experiment the control tested 9.7 per cent, while the average of the three inoculated flasks is 8.55 per cent. The control in the Diplodia experiment of series II tested 9.4 per cent, while the inoculated flasks averaged 1.2 per cent of glucose. Similarly the amount of glucose in the controls and in the flasks in which the organisms have grown may be contrasted with all of those in the same series in all the experiments.

The results show that all these fungi have the power to germinate and grow in a solution of glucose of high osmotic concentration. As early as 1889 Eschenhagen (7) showed that *Aspergillus niger*, *Penicillium glaucum*, and *Botrytis cinerea* Pers. could grow in solutions of glucose of 53, 55, and 51 per cent strength, respectively, and Raciborski (*27*) found that *A. glaucum* and a species of Torula could grow in a salt solution of even greater concentration. Hawkins (*16*) likewise showed that various species

of fungi could thrive in concentrated solutions of glucose ranging from 1.6 to 2.6 molecular and 1.6 and 1.8 molecular sucrose.

With two exceptions the fungi studied by the writers grew on solutions of glucose from approximately 42 to 50 per cent concentration. The two exceptions, *Botrytis cinerea* and *Sphaeronema fimbriatum*, made a fair growth on solutions of approximately 38 and 25 per cent, respectively.

The actual loss in the amount of sugar is somewhat significant and differs considerably with the different fungi. The greatest consumption, if it may be so expressed, occurs in the 10 per cent concentration. In a 10 per cent concentration *Diplodia tubericola* reduced the percentage of sugar from 9.4 to 1.2; *Mucor racemosus* from 9.4 to 5.2; *Sclerotium bataticola* from 9.6 to 2.2; and *Penicillium* sp. from 9.26 to 3.16. In general, the percentage of sugar actually consumed by the organisms just named decreased as the concentration of the solutions increased. On the other hand, *Rhizopus tritici* and *Botrytis cinerea* used more sugar at the higher concentrations, while *Sphaeronema fimbriatum* produced no change.

TOTAL AMOUNT OF GLUCOSE PRESENT

Although the study of Table I shows quite clearly that considerable glucose is converted by the action of some of the fungi, yet this does not show exactly what has occurred, since the figures are expressed in percentages and certain factors are not considered. For example, no account was taken of the amount of evaporation or of the difference in the quantity of solution present in the flasks. Table II shows more accurately the amount of sugar remaining in each flask at the end of the experiment. These figures were obtained by multiplying the number of grams of dextrose in each 100 gm. of solutions—namely, percentage of dextrose present—by the total number of grams of the medium present and dividing the result by 100. The amount of substrate in each flask was determined by deducting the combined weight of the flask and dried fungous growth from that of the solution, flask, and mycelium. In the *Rhizopus tritici* experiment, which was the first one conducted, not enough solution of all strengths was prepared to permit of the addition of 150 cc. to each flask; hence the control flask in some cases contained 100 cc. or even less. This causes what would at first appear to be a great inconsistency in the amount of dextrose present in the control as compared with that in the inoculated flasks. This is especially evident in series VI, where the total amount of dextrose in the control was 41.82, while the average for the inoculated flasks was 78.74. It is, however, also noticeable in series III, IV, and V.

TABLE II.—*Amount of glucose remaining in the solutions at the end of the experiments*

[Expressed in grams]

Series. No.	Percentage of dextrose by weight.	Solution.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus tritici.*	*Mucor racemosus.*
I	0	Control	0	0	0	0
		Inoculated	0	0	0	0
II	10	Control	14. 54	14. 26	12. 96	14. 34
		Inoculated	12. 80	1. 79	12. 33	7. 60
III	20	Control	29. 48	28. 84	23. 39	30. 40
		Inoculated	27. 69	22. 25	26. 94	24. 00
IV	30	Control	45. 50	44. 14	33. 11	46. 69
		Inoculated	43. 78	36. 75	42. 89	40. 71
V	40	Control	63. 48	63. 80	36. 40	64. 85
		Inoculated	62. 34	62. 57	60. 09	61. 43
VI	50	Control	81. 32	85. 34	41. 82	84. 27
		Inoculated	82. 38	85. 25	78. 74	84. 29
VII	60	Control	107. 35	104. 92	100. 90	106. 84
		Inoculated	.....	.....	.....	.....

Series No.	Percentage of dextrose by weight.	Solution.	*Sclerotium bataticola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control	0	0	0	0
		Inoculated	0	0	0	0
II	10	Control	14. 48	13. 72	13. 73	13. 61
		Inoculated	3. 19	4. 53	13. 04	13. 53
III	20	Control	29. 68	28. 89	29. 10	28. 21
		Inoculated	21. 16	23. 37	27. 28	28. 48
IV	30	Control	45. 45	45. 44	46. 14	41. 34
		Inoculated	40. 13	36. 22	41. 50	41. 28
V	40	Control	62. 93	63. 30	63. 62	.....
		Inoculated	60. 74	53. 29	62. 99	.....
VI	50	Control	79. 62	84. 04	84. 05	.....
		Inoculated	79. 25	77. 64	.....	.....
VII	60	Control	.....	105. 96	.....	.....
		Inoculated	.....	104. 62	.....	.....

From Table II it is seen that in no case was all the sugar used up, although the total amount remaining in some cases was small, *Diplodia tubericola*, *Sclerotium bataticola*, and *Penicillium* sp. being the heaviest users.

TOTAL AMOUNT OF GLUCOSE PRESENT AT THE BEGINNING OF THE EXPERIMENTS

In order to determine the amount of glucose utilized by the different fungi it was necessary to calculate the total amount present in each flask at the beginning of the experiments. Data for making these calculations were available, since the total amounts of glucose in the controls were the same at the beginning as at the end of the experiments and the concentrations of the sugar in the inoculated flasks and controls were originally identical.

The figures in Table III show the total amount of glucose present in the control at the beginning of the experiment and the average amount

in the three inoculated flasks of each series. It will be seen that in most cases the amounts in the inoculated flasks averaged very nearly the same as that present in the controls. It must be remembered also that although the solution originally was of a uniform concentration and that 150 cc. were placed in each flask, yet the weight of the contents of the flasks varied slightly, because of the difficulty of measuring accurately with a graduate, which accounts for the difference in the total sugar present.

TABLE III.—*Total calculated amount of glucose present in the solutions at the beginning of the experiments*

[Expressed in grams]

Series No.	Percentage of dextrose by weight.	Solutions.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus tritici.*	*Mucor racemosus.*
I	0	Control	0	0	0	0
		Inoculated	0	0	0	0
II	10	Control	14.54	14.26	12.96	14.36
		Inoculated	14.58	14.25	14.05	14.23
III	20	Control	28.48	28.84	23.39	30.40
		Inoculated	29.42	29.13	29.66	30.39
IV	30	Control	45.50	44.14	33.11	46.69
		Inoculated	45.59	43.79	46.23	45.92
V	40	Control	63.48	63.80	36.40	64.85
		Inoculated	63.15	64.55	64.79	62.42
VI	50	Control	81.32	85.34	41.82	84.27
		Inoculated	81.24	84.78	82.05	84.21
VII	60	Control	107.35	104.92	100.90	106.84
		Inoculated				

Series No.	Percentage of dextrose by weight.	Solutions.	*Sclerotium bataticola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control	0	0	0	0
		Inoculated	0	0	0	0
II	10	Control	14.48	13.72	13.73	13.61
		Inoculated	14.47	13.72	13.81	13.53
III	20	Control	29.68	28.89	29.10	28.21
		Inoculated	29.68	28.84	29.14	28.44
IV	30	Control	45.45	45.44	46.14	41.34
		Inoculated	45.32	43.12	46.15	41.13
V	40	Control	62.93	63.30	63.62	
		Inoculated	62.50	63.23	63.23	
VI	50	Control	79.62	84.04	84.05	
		Inoculated	79.65	82.77		
VII	60	Control		105.96		
		Inoculated		106.38		

TOTAL AMOUNT OF GLUCOSE REDUCED

The amount of glucose present in the solutions both at the beginning and at the end of the experiments having been determined, the differences between these two or the amount reduced by the fungi were calculated and are shown in Table IV. An examination of this table

shows some very interesting results. In discussing these results it must be borne in mind that all the glucose which has disappeared from the solution has not been actually utilized in the formation of the fungous material. Certain compounds, such as alcohol, organic acids, and carbon dioxid probably were formed.

TABLE IV.—*Total amount of glucose reduced*

[Expressed in grams]

Series No.	Percentage of dextrose by weight.	Solution.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus tritici.*	*Mucor racemosus.*
I	0	Control	0	0	0	0
		Inoculated	0	0	0	0
II	10	Control	.....	.....	.....	.....
		Inoculated	1.78	12.45	1.73	6.30
III	20	Control	.....	.....	.....	.....
		Inoculated	1.73	6.89	2.72	6.38
IV	30	Control	.....	.....	.....	.....
		Inoculated	1.81	7.04	3.34	5.18
V	40	Control	.....	.....	.....	.....
		Inoculated	.81	1.67	4.70	2.99
VI	50	Control	.....	.....	.....	.....
		Inoculated	.06	0	3.31	.09
VII	60	Control	.....	.....	.....	.....
		Inoculated	0	0	0	0

Series No.	Percentage of dextrose by weight.	Solution.	*Sclerotium bataticola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control	0	0	0	0
		Inoculated	0	0	0	0
II	10	Control	.....	.....	.....	.....
		Inoculated	11.30	9.18	0.77	.....
III	20	Control	.....	.....	.....	.....
		Inoculated	8.51	5.47	1.85	0
IV	30	Control	.....	.....	.....	.....
		Inoculated	5.18	6.90	4.65	.....
V	40	Control	.....	.....	.....	.....
		Inoculated	1.76	9.60	.25	0
VI	50	Control	.....	.....	.....	.....
		Inoculated	.40	5.15	0	0
VII	60	Control	.....	.....	.....	.....
		Inoculated	0	1.76	.....	.....

In concentrations of approximately 10 per cent, four organisms, *Diplodia tubericola*, *Sclerotium bataticola*, *Penicillium* sp., and *Mucor racemosus*, have reduced a considerable quantity of the total amount of glucose originally present. All these fungi utilize a larger amount of sugar at a strength of 10 per cent than at any higher concentration, except *Penicillium* sp., which reduced slightly more in the 40 per cent solution. Penicillium alone grew and consumed some glucose in an approximately 58 per cent solution. With these organisms *Rhizopus tritici* and *Botrytis cinerea* should especially be compared. The two latter fungi used a comparatively small amount of sugar at any concentration tried. It is, furthermore, significant that they used the lesser amount at the lower concentration, the quantity consumed increasing with the increase of

the concentration up to 40 and 30 per cent, respectively, followed by a reverse of the process.

With the exception of *Penicillium* sp. none of the organisms grew in an approximately 60 per cent solution. Two fungi, *Diplodia tubericola* and *Botrytis cinerea*, would not grow in a 50 per cent solution, and *Sphaeronema fimbriatum* practically not at all at any concentration. It would appear from this table alone that a 10 per cent solution was the optimum concentration for growth of *D. tubericola* and *Sclerotium bataticola;* for *Rhizopus tritici* and *Penicillium* sp., 40 per cent; *B. cinerea*, 30 per cent. *Fusarium acuminatum* and *Mucor racemosus* do about equally well in concentrations of 10, 20, and 30 per cent. Whether or not Table IV gives a fair index of the optimum concentration for growth will be better understood after studying Tables V and VI.

DRY WEIGHT OF MYCELIUM PRODUCED

The dry weight produced by the various organisms in each flask was obtained separately, and the average weight from three flasks of each series is shown in Table V.

TABLE V. *Dry weight of mycelium*

[Expressed in grams]

Series No.	Percentage of dextrose by weight.	Solutions.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus tritici.*	*Mucor racemosus.*
I	0	Control	.....	.....	.....	.....
		Inoculated	0.0150	0.0350	0.0300	0.0131
II	10	Control	.....	.....	.....	.....
		Inoculated	.1094	1.4750	.4716	.3375
III	20	Control	.....	.....	.....	.....
		Inoculated	.1352	1.0692	.6516	.2519
IV	30	Control	.....	.....	.....	.....
		Inoculated	.2049	1.6045	.6464	.1906
V	40	Control	.....	.....	.....	.....
		Inoculated	.1329	.8898	.3660	.1996
VI	50	Control	.....	.....	.....	.....
		Inoculated	0	.1835	.1915	.2433
VII	60	Control	.....	.....	.....	.....
		Inoculated	0	0	0	0

Series No.	Percentage of dextrose by weight.	Solution.	*Sclerotium bataticola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control	.....	.....	.....	.....
		Inoculated	0.0451	0.1682	0.0778	0.0126
II	10	Control	.....	.....	.....	.....
		Inoculated	2.2482	2.6106	.2743	.0191
III	20	Control	.....	.....	.....	.....
		Inoculated	1.8194	2.0308	.9154	.0239
IV	30	Control	.....	.....	.....	.....
		Inoculated	1.4936	2.4063	1.0215	.0103
V	40	Control	.....	.....	.....	.....
		Inoculated	.4858	2.1210	.0811	0
VI	50	Control	.....	.....	.....	.....
		Inoculated	.1114	1.7470	0	0
VII	60	Control	.....	.....	.....	.....
		Inoculated	0	.2717	0	0

In considering the dry weight of mycelium produced, one must bear in mind that the growth of fungi in cultures of the same composition varies greatly. The dry weights given in Table V are the averages of of the growth from three flasks. It frequently happened during the course of these experiments that one culture produced a small amount of mycelium when compared with the other two flasks of the same series. Had it been possible to obtain the average weight from a large number of cultures, the paucity of growth of a single one would not have influenced the average so much.

Kunstmann (*18*) in working with *Aspergillus niger* and *Penicillium glaucum* on sugar solutions found that in extreme cases there were five or more times as much dry material in one culture as in another of the same composition and incubated under identical conditions.

A small amount of mycelium was produced in cultures with no glucose added. *Diplodia tubericola*, *Sclerotium bataticola*, and *Penicillium* sp. produced considerably more dried material than any of the other organisms, especially at concentrations of approximately 10, 20, and 30 per cent; and, as was previously suggested, these were the organisms which utilized in general the largest amount of glucose. *Sphaeronema fimbriatum*, which did not use a determinable amount of sugar, produced a smaller amount of mycelium than any other organism. The dry weight produced in all of the series is, within the limits of experimental error, about equal to the amount produced in the control.

GLUCOSE REQUIRED TO PRODUCE 1 GM. OF DRY WEIGHT

The amount of glucose reduced for each gram of dry weight formed is shown in Table VI. It must be remembered that these figures do not represent necessarily the quantity of glucose which was actually utilized by the fungus in respiration or in producing dry weight, but the amount changed so that it no longer possessed polarizing properties. Doubtless some of the sugar was consumed in forming various compounds resulting from incomplete respiration or other vital activities.

TABLE VI.—*Grams of glucose reduced for each gram of dry weight formed*

Series No.	Percentage of dextrose by weight.	Solution.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus tritici.*	*Mucor racemosus.*
I	0	Control	.....	.....	.....	.....
		Inoculated	0	0	0	0
II	10	Control	.....	.....	.....	.....
		Inoculated	17.52	8.78	3.70	19.65
III	20	Control	.....	.....	.....	.....
		Inoculated	12.60	6.59	4.17	25.56
IV	30	Control	.....	.....	.....	.....
		Inoculated	9.07	4.99	5.17	28.88
V	40	Control	.....	.....	.....	.....
		Inoculated	6.38	2.13	12.91	15.89
VI	50	Control	.....	.....	.....	.....
		Inoculated	.....	.....	17.67	1.44
VII	60	Control	.....	.....	.....	.....
		Inoculated	.....	.....	.....	.....

TABLE VI.—*Grams of glucose reduced for each gram of dry weight formed*—Continued

Series No.	Percentage of dextrose by weight.	Solution.	*Sclerotium bataticola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control	.....	.....	.....	.....
		Inoculated	0	0	0	0
II	10	Control	.....	.....	.....	.....
		Inoculated	5.02	3.58	2.68	.....
III	20	Control	.....	.....	.....	.....
		Inoculated	4.59	2.70	2.07	.....
IV	30	Control	.....	.....	.....	.....
		Inoculated	3.39	2.93	4.56	.....
V	40	Control	.....	.....	.....	.....
		Inoculated	3.86	5.23	5.82	.....
VI	50	Control	.....	.....	.....	.....
		Inoculated	2.98	3.05	.....	.....
VII	60	Control	.....	.....	.....	.....
		Inoculated	.....	8.61	.....	.....

In the same concentration of sugar the different organisms varied greatly in the amount of glucose required to produce 1 gm. of dry weight. Likewise the amount required to produce 1 gm. of dry weight of the same fungus differs greatly with the concentration of the solution. For example, *Fusarium acuminatum* required a little over 6 gm. of glucose to make 1 gm. of dry weight in an approximately 40 per cent solution. On the other hand, in a 10 per cent solution over 17 gm. were required, or nearly three times as much. In other words, the higher the concentration the smaller the amount of glucose used to form 1 gm. of dry weight. Within the limits of these experiments the "economic coefficient" of *F. acuminatum* in a 40 per cent solution would be 6.38. *Diplodia tubericola* forms a curve similar to that of *F. acuminatum*, but the quantity of glucose required to produce 1 gm. of dry weight is considerably less in corresponding concentrations of the solution, its "economic coefficient" in about the same concentration being 2.13. *Rhizopus tritici* reverses the process, the lowest "economic coefficient" of 3.70 being in an approximately 10 per cent concentration. In a 50 per cent solution the same fungus utilized 17.67 gm. of glucose to produce 1 gm. of dry material. If *R. tritici* is compared with *Mucor racemosus*, which required 1.44 gm. of sugar to produce 1 gm. of dry weight, it is seen that the order is again reversed, the lowest "economic coefficient" occurring in a concentration of 50 per cent. The highest "economic coefficient" of the latter organism was in a 28.7 per cent solution and increased both above and below this concentration. *Sclerotium bataticola* parallels *M. racemosus* and in every case except in a 50 per cent solution requires much less sugar to form 1 gm. of dried material. Neither *Penicillium* sp. nor *Botrytis cinerea* is quite consistent, in that the minimum glucose required per gram of dry weight is not at either end of the series.

A comparison of the maximum amount of glucose required to form 1 gm. of dry weight shows remarkable variations among the different

fungi. *Mucor racemosus*, requiring 28.88 gm., stands at one extreme and *Sclerotium bataticola*, requiring 5.02 gm., at the other. *Fusarium acuminatum*, *Diplodia tubericola*, and *Rhizopus tritici* required 17.52, 8.78, and 17.67 gm., respectively. Pfeffer (*25*) and Kunstman (*18*) have termed the numerical relation between the sugar consumed and the dry weight of the substance formed the "economic coefficient." The minimum amount of sugar required to produce 1 gm. of dry weight shows likewise great variation. A comparison of *F. acuminatum* and *M. racemosus*, the two extremes, shows that the "economic coefficient" of the former is about 4.4 times greater than that of the latter. These differences correspond fairly well with results obtained by Kunstmann working with *Aspergillus niger* and *Penicillium glaucum*, who found it to vary from 1.13 to 3.88, while Ono (*24*) obtained a value as high as 6.1 when working with *A. niger*.

These results indicate that the coefficient cannot be looked upon as in any way constant. It differs with different fungi grown under identical conditions. There may be several factors which influence the results. According to Jost (*17*), the coefficient increased with the progressive development of the fungus and with the elevation of the temperature. Toxic substances likewise were shown by Ono to increase the coefficient in Aspergillus. Since these organisms were all grown at the same temperature, it would be largely eliminated as a controlling factor. On the other hand, some of the chemicals or impurities composing the nutrient medium might have a toxic action on some of the organisms. Furthermore, it is likely that the different fungi vary greatly in their ability to form other substances, such as alcohol, organic acids, etc., which might be toxic or inhibit normal growth. In some cases it has been shown that alcohol is actually produced. That all organisms are not equally influenced by these changes is probably true. In the more resistant forms growth would continue for a longer time and thus possibly produce a greater amount of fungal material per unit volume of sugar. What changes the fungus actually brings about must remain unknown until chemical methods permit of the quantitative determinations of the different products and materials formed.

HYDROGEN-ION CONCENTRATIONS

The hydrogen-ion concentrations express in P_H values were determined from representative samples of all the solutions. These are recorded in Table VII.

TABLE VII.—*Hydrogen-ion concentrations, expressed in terms of potential hydrogen*

Series No.	Percentage of dextrose by weight.	Solution.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus tritici.*	*Mucor racemosus.*
I	0	Control	4.12	4.41	6.33	6.54
		Inoculated	4.24	3.89	6.12	6.02
II	10	Control	3.97	3.97	4.84	5.44
		Inoculated	3.89	3.66	2.11	3.09
III	20	Control	3.93	3.89	4.50	5.06
		Inoculated	3.71	2.52	1.90	3.21
IV	30	Control	3.96	3.91	4.20	4.30
		Inoculated	3.66	2.30	1.81	3.46
V	40	Control	4.22	3.75	4.10	4.39
		Inoculated	3.62	2.27	2.00	3.13
VI	50	Control	3.97	4.07	4.00	4.28
		Inoculated	3.99	3.84	2.81	4.17
VII	60	Control	4.09	3.72	4.12	4.08
		Inoculated	.....	.....	.....	.....

Series No.	Percentage of dextrose by weight.	Solution.	*Sclerotium batalicola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control	6.49	6.44	6.39	6.41
		Inoculated	5.15	6.41	6.25	6.41
II	10	Control	4.54	5.70	4.99	5.19
		Inoculated	4.21	4.37	3.24	5.50
III	20	Control	4.83	5.39	4.51	5.16
		Inoculated	4.06	3.81	2.91	5.05
IV	30	Control	4.80	5.14	4.22	4.72
		Inoculated	4.07	3.92	3.45	4.83
V	40	Control	4.82	4.79	4.07	4.52
		Inoculated	3.69	3.44	3.92	.....
VI	50	Control	4.76	4.51	4.00	.....
		Inoculated	4.75	3.29	.....	.....
VII	60	Control	4.75	4.40	.....	.....
		Inoculated	.....	3.16	.....	.....

No attempt was made to bring the different solutions to a definite or uniform hydrogen-ion concentration. What influence the organisms had on the hydrogen-ion concentration was determined by a comparison of the controls with the solutions after the removal of the fungous growth. That the solutions differed somewhat in their original hydrogen-ion concentration is seen from Table VII. Gillespie (*11*) in working with different strains of the potato scab organism, *Actinomyces chromogenus* Gasp. in culture media of different composition found that the growth was slower and less vigorous in a solution with a hydrogen-ion concentration of P_H 5.2 than in less acid media. It would seem that, although there was some variation as regards the tolerance of the different strains to acid media, a P_H of 5.2 would closely approximate the limit of growth. A further interesting fact in this connection also was brought out by Gillespie, who showed that cultivated soils of the Caribou loam, which is generally free from the scab organism, yield a water extract with P_H values varying from 4.9 to 5.5 with a mean of 5.2. Meacham (*22*) found

that the growth of *Lenzites sepiaria* (Wulf.) Fr., *Fomes roseus* (Alb. and Schw.) Fr., *Coniophora cerebella* (Pers.) Schröter, and *Merulius lachrymans* (Jcq.) Fr. in synthetic and malt extract media was not inhibited until a high hydrogen-ion concentration was reached. The limiting P_H value was found to be near 1.7. All the fungi responded in about the same way, although there were distinct variations between the different organisms. Growth appeared to be retarded when a P_H value of about 3.0 was reached. As the acidity increased beyond this point the growth became markedly less.

Webb (*29*) found that in a culture solution consisting of mannite, phosphoric acid, and sodium hydroxid successively increasing the hydrogen-ion concentrations from approximately neutral to P_H 3.1 to 2.8 influenced favorably the germination of the spores of *Aspergillus niger*, *Penicillium cyclopium* Westl., *Botrytis cinerea*, *Fusarium* sp., and *Lenzites sepiaria*. The different organisms had a different range of hydrogen-ion concentration, permitting the germination of the spores as follows: *A. niger*, P_H 2.8 to 8.8; *P. cyclopium* 2.8 to 10+; *B. cinerea* 2.8 to 7; *Fusarium* sp., 2.8 to 10+; and *L. sepiaria* 2.8 to 7.

At certain concentrations some of the organisms studied by the writers had little or no influence on the acidity of the substrate, while others rendered it much more acid. For example, *Fusarium acuminatum*, *Sclerotium bataticola*, and *Sphaeronema fimbriatum* can hardly be interpreted as exercising any influence on the acidity of the solution within the limits of these experiments. With all three of these fungi the hydrogen-ion concentration in the control at the same concentrations is actually higher than the solution on which the fungus grew. The differences are slight and in some cases well within the limits of experimental error, so that in general there are no outstanding examples of increase or decrease in acidity of any one of these three fungi. On the other hand, the remaining five organisms all show a considerable increase in the hydrogen-ion concentration in solutions of different strength. *Rhizopus tritici*, for example, increased the acidity of the substrate from P_H 4.2 to 1.81 in a solution of 30 per cent dextrose, when the maximum hydrogen-ion concentration was reached. In general, it may be said that the highest concentration was reached in solutions containing about 20 to 30 per cent glucose and that it decreased above and below these strengths.

Just how nearly these results represent the limits of growth for these fungi is not definitely known. The greatest growth was made during the first week; thereafter it slowed up. Whether or not this was due to the increase of acidity in the solution or to other causes can not be answered at the present time.

Preliminary experiments showed that if *Rhizopus tritici* is grown several times on the same solution after removal of the fungus felt it finally reaches a stage when growth no longer will take place. These experiments were made with Czapek's modified nutrient solution with a 2 and 1 per

cent of glucose and starch, respectively, as a source of carbon and with sweet potato bouillon. The fungus was allowed to grow 10 days, when the felt was removed and the dry weight determined. The Czapek's nutrient solution had a hydrogen-ion concentration of P_H 3.07 previous to inoculation. After a 10 days' growth the acidity had increased to P_H 2.05. The solution was sterilized by autoclaving and was then reinoculated with the same organism, but no growth resulted. Repeated inoculations were made, but no growth resulted. No appreciable increase or decrease in the P_H value of the solution resulted from the autoclaving either in Czapek's solution or in the sweet potato bouillon. Although the fungus would not grow, there remained a considerable quantity of glucose and starch in the solution. In a 2-liter flask containing 1,000 cc. of solution 1.28 gm. of dried mycelium were produced.

The sweet potato bouillon had an original P_H value of 5.17, which was increased by a 10 days' growth to 3.12. The dry weight produced on 1,000 cc. of solution in a 2-liter flask amounted to 3.59 gm. The solution was sterilized by autoclaving and was reinoculated. At the end of 10 more days the P_H value was 3.08 and the dry weight was 1.33 gm. This operation was twice more repeated, and the P_H values after two more periods of 10 days' growth were 3.11 and 3.05, respectively. The dry weight of the mycelium in the former case was 0.2536 gm., and not enough was produced in the latter to determine. Glucose and starch still remained in the solution. It is seen that the hydrogen-ion concentration did not increase materially after the first 10 days' growth, while the actual amount of dry weight of material greatly decreased.

OSMOTIC CONCENTRATIONS OF THE SOLUTIONS

Table VIII shows the osmotic pressures in atmospheres of the sugar solutions at the end of the experiments. These values were determined for the first five series of each organism. There was so little growth in the highest concentrations that they were not considered of sufficient importance to justify the determination of their osmotic pressures.

Attention was previously called to the fact that fungi can germinate and grow in a sugar or salt solution far more concentrated than that of the cell sap of its host. The literature is replete with instances of certain fungi, such as species of Penicillium and Aspergillus, which are able to grow on concentrated sugar solutions. All the organisms discussed in this paper will germinate on a solution of glucose with an osmotic concentration several times greater than that of the cell sap of the sweet potato.

An examination of Table VIII shows that these fungi grew in solutions with a maximum osmotic pressure varying from 81.33 to 101.46 atmospheres. It will be noticed also that in general *Fusarium acuminatum* and *Mucor racemosus* increased, while all the other organisms decreased the osmotic concentration of the solutions during a period of two weeks' growth. Some exceptions in this connection should be noted. *F.*

acuminatum decreased the osmotic concentration in Czapek's modified nutrient solution without sugar added more than any of the other organisms. *Sclerotium bataticola* and *Botrytis cinerea* decreased the concentrations in all except 40 per cent solutions, where there was a slight increase. The decrease in the osmotic pressure is especially marked with *S. bataticola* and *Penicillium* sp. in the 10 per cent solution, being reduced in the former from 16.12 to 9.31 atmospheres and in the latter from 16.48 to 6.26 atmospheres.

TABLE VIII.—*Osmotic pressure of the sugar solutions*

[Expressed in atmospheres]

Series No.	Percentage of dextrose by weight.	Solutions.	*Fusarium acuminatum.*	*Diplodia tubericola.*	*Rhizopus tritici.*	*Mucor racemosus.*
I	0	Control	2.41	2.17	1.93	2.29
		Inoculated	1.93	2.01	1.81	2.29
II	10	Control	16.37	16.24	16.36	16.84
		Inoculated	17.24	12.52	14.44	19.04
III	20	Control	.....	33.36	34.79	35.75
		Inoculated	.....	31.09	32.41	39.53
IV	30	Control	66.76	53.37	62.03	59.30
		Inoculated	68.73	53.17	58.27	63.52
V	40	Control	101.46	92.20	98.12	93.85
		Inoculated	101.89	92.59	95.64	98.51
VI	50	Control	.....	.....	.....	.....
		Inoculated	.....	.....	.....	.....
VII	60	Control	.....	.....	.....	.....
		Inoculated	.....	.....	.....	.....

Series No.	Percentage of dextrose by weight.	Solutions.	*Sclerotium bataticola.*	*Penicillium* sp.	*Botrytis cinerea.*	*Sphaeronema fimbriatum.*
I	0	Control	2.17	1.93	1.93	1.93
		Inoculated	2.13	1.67	1.89	1.85
II	10	Control	16.12	16.48	15.76	15.88
		Inoculated	9.31	6.26	14.84	15.96
III	20	Control	33.96	34.68	33.36	33.60
		Inoculated	30.89	27.86	31.45	33.72
IV	30	Control	55.03	58.47	58.00	52.06
		Inoculated	54.04	48.02	54.75	52.01
V	40	Control	87.98	81.33	90.68	81.62+
		Inoculated	90.92	80.39	92.12	.....
VI	50	Control	.....	.....	.....	.....
		Inoculated	.....	.....	.....	.....
VII	60	Control	.....	.....	.....	.....
		Inoculated	.....	.....	.....	.....

Why some organisms increase and others decrease the osmotic concentration of the solution is not definitely known. It is difficult to explain why an organism decreases the osmotic pressure in all strengths of the sugar solution except in the 40 per cent and there slightly increases it. In this connection *Sclerotium bataticola* and *Botrytis cinerea* should be especially noted.

In seeking for an explanation of the increase or decrease of the osmotic concentration by the different organisms in solutions containing the same percentage of sugar, one should take into consideration the cleavage products formed. Many investigators have shown that some fungi, and it may apply to all, have the power to produce organic acids, alcohol, etc. Wehmer (*30*) gave the generic name Citromyces to a group of fungi which he believed to be characterized by their ability to produce citric acid. Oxalic acid fermentation was thought by him to be a characteristic of *Aspergillus niger*. Currie (*4*) some years later showed that citric acid was likewise produced by *A. niger* in nutrient solutions.

Lind (*21*) showed that oxalic acid was produced by *Aspergillus niger*, *Penicillium glaucum*, and *Botrytis cinerea*, and Lafar (*19, p. 351*) by *A. niger*. Lafar also found that citric acid was formed by *Citromyces pfefferianus* Wehm., *C. glaber* Wehm., and *P. luteum*. Many other references might be cited to show that a great variety of fungi and bacteria produce acids of various sorts.

The production of alcohol has been proved to be a regular phenomenon of many fungi, the amount formed depending on the substrate, the temperature at which incubated, and the length of the incubation period.

Gayon (*10*), Hansen (*12*), Fitz (*9*), and Brefeld (*2*) showed that *Mucor spinosus* v. Tieg. and *Rhizopus nigricans* produced alcohol in varying amounts, depending somewhat upon the conditions mentioned above. *M. racemosus* produced alcohol but little when incubated at a temperature below 15° C. *R. nigricans* ceased to produce alcohol when 1.5 per cent had been formed. *M. spinosus*, according to Gayon, will produce as much as 1.5 to 2 per cent, and *M. erectus* Bain. as much as 8 per cent, according to Hansen, when incubated at room temperature. If either alcohol or acids or both were formed in the solutions the osmotic concentration would be influenced in proportion to the amount produced. Previous tables have shown that the percentage of sugar was lowered by the fungus, and yet in spite of that the osmotic concentration of some of the solutions is actually higher than that of the controls. This would be possible only in case some of the sugar or other constituents of the substrate were converted into some substance, such as alcohol or organic acids, which would affect the osmotic concentration. In *M. racemosus* the osmotic pressure of the solution of the inoculated flask is higher than in the control, although a considerable amount of the sugar was actually removed. This organism is one which is known, as pointed out in one of the citations given above, to produce a considerable quantity of alcohol. Data presented in Table II show that *Diplodia tubericola*, *M. racemosus*, and *Sclerotium bataticola* growing in an approximately 10 per cent solution reduced the sugar from 14.26, 14.34, and 14.48 to 1.29, 7.60, and 3.18 gm., respectively. On the other hand, the osmotic pressures of these solutions were changed from 16.24, 16.84, and 16.12

to 12.52, 19.04, and 9.31, respectively. In the first and last cases the osmotic pressure is reduced, the latter more than the former, but in neither case in proportion to the amount of sugar actually disposed of. In *M. racemosus*, which reduced the sugar by nearly one-half, the osmotic pressure was actually increased. A study of the two tables will show other similar results. These facts may be explained in part by assuming that some of the sugar was broken down into some substance or substances which held the osmotic concentration up to, or in some cases raised it above, that of the control.

CHARACTER OF MYCELIAL GROWTH

The fungi all looked very much alike when grown upon the culture medium without glucose present. The mycelium was white or grayish in color, very fine, and usually formed a thin felt over the surface with a small amount of submerged threads. Except in *Rhizopus tritici*, no sporulation took place, and then only an occasional sporangium was formed. With two exceptions, all the fungi studied made a fairly good growth in concentrations of glucose varying roughly from 10 to 40 per cent. Sphaeronema apparently is not able to utilize glucose, since in no case was the growth perceptibly increased by its presence. Of the eight fungi, Penicillium alone was able to grow in the strongest concentration. *Diplodia tubericola*, *R. nigricans*, *Mucor racemosus*, and *Sclerotium bataticola* made some growth in all but series VII, while *Fusarium acuminatum* made a sparse growth in series VI. *Sphaeronema fimbriatum* and *Botrytis cinerea* failed to thrive in series V and VI, respectively. Although Penicillium grew at the highest concentration used, it did not develop normally. No doubt this strength of dextrose represents approximately the limit of growth of this organism.

SUMMARY

(1) Eight fungi—*Fusarium acuminatum*, *Diplodia tubericola*, *Rhizopus tritici*, *Mucor racemosus*, *Sclerotium bataticola*, *Penicillium* sp., *Botrytis cinerea*, and *Sphaeronema fimbriatum*—which cause decay of sweet potatoes in storage, were grown at a constant temperature of 28° C. on a modification of Czapek's nutrient solution, with different amounts of glucose as a source of carbon. All these fungi except *S. fimbriatum* utilized glucose in considerable amounts.

(2) The different fungi varied greatly in the amount of glucose they actually consumed at the same concentration. In general, the greatest consumption was in the weaker solution (10 per cent) and decreased progressively with the increase of the concentration. With two exceptions, all the organisms grew in solutions containing from 42 to 50 per cent glucose. *Penicillium* sp. alone grew in a 58 per cent solution.

(3) A great variation was found among the different fungi in the amount of dry material that was produced at the same concentration.

The concentration on which the greatest yield of fungous material was produced by a certain organism was not necessarily the optimum concentration for any of the other fungi.

(4) The different organisms varied greatly in the amount of glucose required to produce 1 gm. of dry weight. Likewise the amount required to produce 1 gm. of dry weight of the same fungus differed with the concentration of the solution.

(5) The "economic coefficient" was found to be much higher in many cases than that given by Kunstmann and Ono; the maximum of 28.88 and the minimum of 1.44 being reached by *Mucor racemosus* on a 30 and 50 per cent solution, respectively. The highest "economic coefficient" for some fungi was on the weaker solutions. For other organisms, however, the order was reversed.

(6) Some of the organisms—namely, *Fusarium acuminatum*, *Sclerotium bataticola*, and *Sphaeronema fimbriatum*—had little or no influence on the hydrogen-ion concentration. *Rhizopus tritici*, *Diplodia tubericola*, *Mucor racemosus*, *Penicillium* sp., and *Botrytis cinerea*, on the other hand, increased perceptibly the acidity of the solution.

(7) All of the fungi studied grew in solutions with a maximum osmotic pressure varying from 81.33 to 101.46 atmospheres. *Fusarium acuminatum* and *Mucor racemosus* increased the concentration, whereas, the other fungi in general decreased it. In a few cases where a considerable amount of sugar was consumed the concentration was actually increased. In general, the decrease in the osmotic concentration was not in proportion to the sugar consumed, so that it is possible that compounds such as organic acids, alcohol, etc., were formed from the sugar which would themselves influence the osmotic concentration.

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RESPIRATION OF SWEET POTATO STORAGE-ROT FUNGI WHEN GROWN ON A NUTRIENT SOLUTION

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INTRODUCTION

Respiration in plants has been a favorite subject for botanical research for many years. Most of the earlier work was done with chlorophyll green plants, or parts of plants, some of the classical studies having been made upon seeds. The results of these researches have given us a better insight into the metalbolism of flowering plants, with some facts regarding the utilization of carbohydrates in respiration. Hasselbring and Hawkins (*9*)[1] in studying the respiration of the roots of sweet potatoes found that the reducing sugars were the immediate source of respiratory material. The cane sugar remained relatively stable when once formed and did not appear to be readily utilized so long as starch and other carbohydrates were present in abundance.

The results of investigations reported by the writers (*23*) show that the fungi responsible for most of the decay of sweet potatoes—namely *Fusarium acuminatum* E. and E. emend. Wollenw., *Sclerotium bataticola* Taub., *Diplodia tubericola* (E. and E.) Taub., *Penicillium* sp., *Mucor racemosus* Fes., *Botrytis cinerea* Pers., *Rhizopus tritici* Saito, and *Sphaeronema fimbriatum* (E. and H.) Sacc.—with the exception of the last-named organism, can utilize glucose as a source of carbon. Not only were these fungi able to use glucose in various amounts, but they also produced certain changes in the hydrogen-ion and osmotic concentrations of the culture medium. Some of the sugar was used in producing mycelium and in supplying energy for carrying on the vital processes of the organisms, although no doubt a part of it was utilized for other purposes.

The investigations reported in this paper were designed to throw further light (1) upon the question of the availability of glucose as a source of carbon for these same fungi, *Sphaeronema fimbriatum* excepted, and (2) upon the amount of the carbohydrate used in respiration as measured by the amount of carbon dioxid (CO_2) given off.

METHODS

In these investigations the fungi studied were grown in Erlenmeyer flasks on a liquid synthetic medium. The apparatus was set up in duplicate,

[1] Reference is made by number (italic) to "Literature cited," p. 225–226.

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and parallel experiments were run in most cases. The culture flasks were placed in an incubator held at a constant temperature, and CO_2-free air was pulled through by means of a Richard air pump. The air was passed through three bottles containing pumice stone and concentrated potassium hydroxid (KOH) and one bottle containing concentrated barium hydroxid ($Ba(OH)_2$), the latter being used principally as an indicator to insure that the air was entirely freed of CO_2. The air was finally washed by being pulled through CO_2-free distilled water, from which it was drawn into the culture flasks. The culture flasks and trap bottles for freeing the air of CO_2 and for washing it were placed in an incubator maintained at a temperature of 29° C. From the culture flasks the air was drawn out of the incubator through glass tubing. It was then freed of CO_2 by being drawn through a series of cylinders containing a saturated solution of $Ba(OH)_2$. Fresh air, though not in sufficient amount to interfere with the temperature control, was admitted to the incubator through a small hole about 1 cm. in diameter.

The cultures were grown in 1-liter Jena Erlenmeyer flasks stoppered with 2-holed rubber corks, through each of which were passed two glass tubes which served for the exchange of air. The tubes which admitted the air into the flasks extended to within about 1 inch of the surface of the medium, while the exit tubes barely projected through the corks.

CULTURE MEDIUM

A modification of Czapek's nutrient solution, in which ammonium nitrate (NH_4NO_3) was substituted for sodium nitrate ($NaNO_3$), was used as a substratum. Baker's C. P. dextrose in approximately 10 per cent strength was supplied as a source of carbon. The culture medium was prepared according to a method fully discussed in an article by Weimer and Harter (*23*), to which the reader is referred for complete details.

PREPARATION OF AN EXPERIMENT

Three 1-liter flasks, one to serve as a control, were prepared to contain 300 cc. of the culture medium. After being plugged with cotton they were sterilized by steaming for 20 minutes on three consecutive days. Just before inoculation the cotton plug was replaced by the rubber stopper with glass tubes attached, and the whole was resteamed for 20 minutes. To allow for the expansion on heating and to prevent contamination on cooling a glass tube of 3-mm. bore, about 8 cm. long, with a small bulb about 1 cm. in diameter midway between the two ends, was connected with each of the tubes passing through the cork by means of rubber tubing. The bulb was packed with cotton, which while permitting the passage of air served to filter out all contaminating material. The cotton was left in the bulb throughout the experiment.

Inoculations were made with a loop of a heavy spore suspension in distilled water or with a bit of mycelium from a young and vigorous cul-

ture growing on Irish potato cylinders or stems of *Melilotus alba* Desr. Before the culture flasks were inserted into the apparatus, air was pulled through the series of trap bottles and cylinders for about one hour to make sure (1) that all of the CO_2 was being removed from the incoming air and (2) that there was no leak through the connections.

At the termination of each experiment the mycelium was filtered into a tared alundum crucible washed with distilled water and dried to constant weight in a vacuum oven at 60° C. The percentage of glucose present in the control as well as that remaining in the two culture flasks was determined by a Fric saccharimeter, from which the total amount reduced was calculated. The culture and control flasks were weighed at the beginning and at the close of the experiment, and from these data corrections were made for loss of water due to evaporation or other causes.

DETERMINATION OF CO_2

The fungi differed markedly in the rapidity at which they grew in the culture solution. Some of the organisms, as *Rhizopus tritici*, for example, grew rapidly from the outset, and CO_2 was evolved in from two to three days. Some of the other fungi, on the other hand, grew slowly and gave off no CO_2 for a week or more. As soon as a precipitate appeared in the $Ba(OH)_2$ solution the CO_2 was determined for each 24-hour period thereafter to the end of the experiment.

In the determination of the CO_2 evolved the excess $Ba(OH)_2$ was neutralized by the addition of hydrochloric acid (HCl), with the use of thymol blue (thymol sulphonphthalein) as an indicator. The precipitate ($BaCO_3$) was then dissolved by adding an excess of *N/1* HCl. After the total volume of the solution had been determined, an aliquot portion (usually 25 cc.) was titrated against *N/10* sodium hydroxid (NaOH) with brom phenol blue (tetra bromo phenol sulphonphthalein) used as the indicator. The number of cubic centimeters of *N/10* NaOH used to neutralize 25 cc. of the solution was multiplied by the total volume of solution, which gives the equivalent of the excess acid. The excess acid was then converted into its equivalent of *N/1* HCl, and this amount was deducted from the total number of cubic centimeters of HCl required to dissolve the precipitate. The figure thus obtained was multiplied by the factor 0.022, the equivalent in CO_2 of 1 cc. of *N/1* HCl.

In the preliminary experiments the two indicators, phenolphthalein and methyl orange, usually used in titrations of this nature were tried, but neither gave a satisfactory end point. Scales (*18*) experienced like difficulties in titrations of a similar kind and found that thymol blue and brom phenol blue both had a very sharp end point. Thymol blue in the presence of $Ba(OH)_2$ and $BaCO_3$ gives a brilliant blue color, which changes to a muddy green at the neutral point, to a lemon-yellow in a slight excess of acid, and to a pink in a strong acid solution. Upon the

addition of brom phenol blue the solution changes to a deep blue color and when slightly acid to a lemon-yellow.

EXPERIMENTAL DATA

RATE OF RESPIRATION

The rate of respiration of the different fungi expressed in CO_2 production is shown by the curves in figure 1, where the abscissae represent days and the ordinates the amount of CO_2 produced daily in grams.

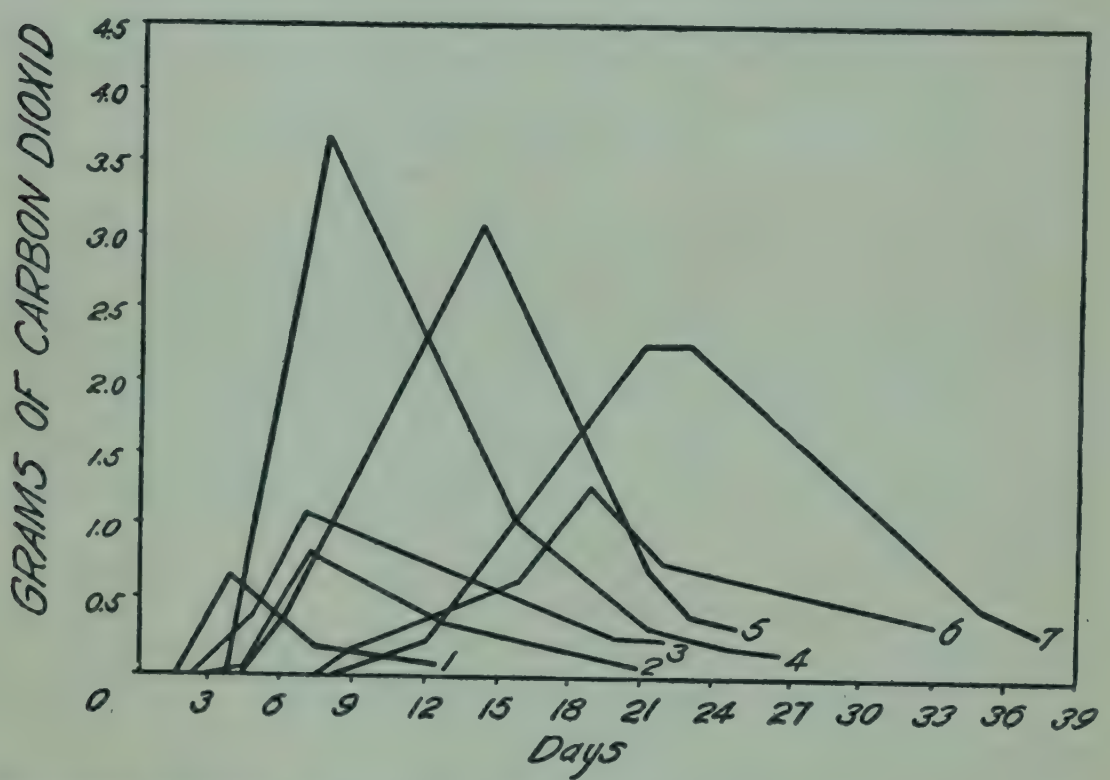

FIG. 1.—Rate of respiration of various fungi: 1, *Rhizopus tritici;* 2, *Diplodia tubericola;* 3, *Mucor racemosus;* 4, *Penicillium* sp.; 5, *Sclerotium bataticola;* 6, *Fusarium acuminatum;* 7, *Botrytis cinerea.*

The figures used in plotting these curves are in most cases the averages of two parallel tests. One familiar with the physiology and growth of fungi is aware that the same nutrient medium, the same temperature, and other environmental factors do not meet the requirements of all fungi equally well. In order to make the results comparable, a uniform standard of conditions for all the organisms was established as nearly as possible. They were all grown in an incubator maintained at a constant temperature of 29° C. in the dark.

An inspection of the curves shows that three organisms—namely *Penicillium* sp., *Botrytis cinerea*, and *Sclerotium bataticola* produced a quantity of CO_2 in excess of 2 gm. in a single day, while the four remaining organisms produce a relatively small amount. The data show also that those organisms which grew rapidly produced a comparatively small amount of CO_2, reaching their maximum in a short time after the culture flasks were inoculated and declining steadily thereafter. *Rhizopus*

triiici illustrates this point. On the second day it produced a measurable amount of CO_2, the quantity steadily increasing up to the fourth day. Thereafter it gradually decreased and practically ceased entirely by the twelfth day. *Penicellium* sp., *B. cinerea*, and *S. bataticola*, on the other hand, grew slowly and did not give off a measurable quantity of CO_2 for several days following inoculation. The evolution of CO_2 rapidly increased thereafter and continued a considerable number of days before it ceased entirely.

Except with *Botrytis cinerea*, the day of maximum production of CO_2 was followed by a sharp decline, which continued to the close of the experiment. *B. cinerea* differed from all the other organisms in that there was a period of two or three days when the maximum CO_2 production remained about constant. However, it is probable that if determinations had been made in less than 24-hour periods an apex in the curve would have been shown for this fungus.

DRY WEIGHT OF MYCELIUM

A direct comparison of the dry weight of the different organisms is not possible for reasons that can be well understood. As previously pointed out, the fungi were allowed to grow as long as a measurable quantity of CO_2 was given off. This varied from 11 to 30 days, so that some fungi had a much longer time to form mycelium. Furthermore, some fungi will produce a much larger quantity of dried material than others under identical conditions; in fact, it was shown by Kunstmann (*14*) that in duplicate cultures of the same fungus two and one-half times as much dried material might be produced in one flask as in another.

An examination of Table I and the curves in figure 1 will show that although the experiments with *Botrytis cinerea* ran for 38 days, *Penicillium* sp. produced about 11 per cent more dry weight in 10 days less time and that *Sclerotium bataticola* produced a greater dry weight in 25 days than *B. cinerea*, which grew 12 days longer. *Mucor racemosus* and *Rhizopus tritici* were grown 23 and 12 days and produced 1.13 and 0.94 gm. of dry weight, respectively. A comparison of *M. racemosus* with *S. bataticola* shows an even more striking difference, the ratio of dry weight being approximately 1 to 5.5.

TABLE I.—*Glucose reduced and dry weight and CO_2 formed by certain sweet potato storage-rot fungi, together with the numerical expression of the interrelationship existing between the glucose changed and the resulting products as well as between the products themselves*

Organism.	Solution.	Dry weight of mycelium produced (in grams).	Glucose remaining at the end of the experiment (in percentage by weight).	Amount of glucose reduced (in grams).	Total amount of CO_2 (in grams).
Fusarium acuminatum	Control	.....	9.2	.....	.....
	Inoculated	1.607	.55	27.510	15.524
Sclerotium bataticola	Control	.....	9.18	.....	.....
	Inoculated	6.068	.09	27.771	29.327
Diplodia tubericola	Control	.....	9.1	.....	.....
	Inoculated	1.972	6.35	7.628	6.066
Penicillium sp	Control	.....	9.2	.....	.....
	Inoculated	6.251	0	30.791	32.523
Mucor racemosus	Control	.....	9.1	.....	.....
	Inoculated	1.127	1.5	25.762	12.837
Botrytis cinerea	Control	.....	9.6	.....	.....
	Inoculated	5.377	.05	27.271	32.714
Rhizopus tritici	Control	.....	9.2	.....	.....
	Inoculated	.938	7.93	4.345	2.718

Organism.	Solution.	Grams of CO_2 given off per gram of glucose reduced.	Dry weight of mycelium (in grams produced by 1 gm. of glucose).	Coefficient of respiration: sugar (in grams reduced per gram CO_2 formed).	Economical coefficient: sugar used (in grams to produce 1 gm. dry weight).	Respiratory quotient: dry weight (in grams formed per gram CO_2 given off).
Fusarium acuminatum	Control	.....	.....	.....	.....	.....
	Inoculated	0.567	0.058	1.77	17.11	0.103
Sclerotium bataticola	Control	.....	.....	.....	.....	.....
	Inoculated	1.056	.218	.95	4.58	.207
Diplodia tubericola	Control	.....	.....	.....	.....	.....
	Inoculated	.795	.258	1.26	3.86	.325
Penicillium sp	Control	.....	.....	.....	.....	.....
	Inoculated	1.056	.203	.95	4.93	.192
Mucor racemosus	Control	.....	.....	.....	.....	.....
	Inoculated	.498	.044	2.01	22.86	.088
Botrytis cinerea	Control	.....	.....	.....	.....	.....
	Inoculated	1.200	.197	.83	5.07	.164
Rhizopus tritici	Control	.....	.....	.....	.....	.....
	Inoculated	.625	.216	1.59	4.63	.345

UNCHANGED GLUCOSE REMAINING IN THE SOLUTION

With the exception of *Diplodia tubericola* and *Rhizopus tritici* the fungi either utilized directly practically all the glucose originally present in the solution or converted it into other substances (Table I, columns 4 and 5), the former utilizing about 2.8 and the latter 1.3 per cent. The percentage of glucose consumed should be carefully compared with the dry weight of mycelium shown in column 3. *Fusarium acuminatum* and *Sclerotium bataticola* utilized all but 0.55 and 0.09 per cent, respectively, of the glucose and produced 1.61 and 6.1 gm. of mycelium. In

other words, *S. bataticola* made nearly four times as much dry weight in nine days less time by the utilization of practically the same amount of sugar as *F. acuminatum*. *Penicillium* sp., on the other hand, utilized all the glucose and formed 6.25 gm. of mycelium, while *Mucor racemosus* consumed 25.8 gm. of sugar in making 1.13 gm. of dry weight. A study of the figures shows that there is no uniformity among the fungi as regards the glucose utilized and dry weight produced, even if an account is taken of the length of time they were grown.

CO_2 PRODUCTION

In column 6 is shown the total amount of CO_2 produced by each fungus during the time of the experiment. A comparison of the total CO_2 given off with the dry weight produced and sugar utilized will not be necessary in all cases, but attention will be called to a few outstanding examples. *Sclerotium bataticola* gave off nearly twice as much CO_2 as *Fusarium acuminatum* and yielded nearly four times as much dry weight, while *Botrytis cinerea* and *Rhizopus tritici* produced 32.71 and 2.72 gm. of CO_2, respectively, and formed 5.38 and 0.94 gm. of dry weight. Here also a résumé of the figures shows that there is little or no uniformity in the relation of CO_2 evolved to the glucose consumed and dry weight produced.

RELATION OF CO_2 GIVEN OFF TO GLUCOSE REDUCED

The amount of CO_2 given off in grams for every gram of sugar reduced is shown in column 7. It will be seen that for *Sclerotium bataticola*, *Penicillium* sp., and *Botrytis cinerea* more than 1 gm. of CO_2 is produced for each gram of sugar consumed. In all the other cases it is less than unity. It should be noted that these are the three fungi which produced the largest amount of dry weight and are among those which consumed the most glucose. If extremes are contrasted it will be seen that *B. cinerea* and *Mucor racemosus* produce approximately 1.2 and 0.5 gm. of CO_2, respectively, for each gram of sugar utilized. While some reasons for these differences will be suggested later, attention should be called to the fact that *B. cinerea* continued to give off an appreciable amount of CO_2 some days longer than *M. racemosus*. Botrytis, Penicillium, and Sclerotium consumed all or practically all the sugar, so that it is not unlikely that other compounds were formed from the sugar which may have been utilized as a source of carbon.

RELATION OF DRY WEIGHT TO GLUCOSE CONSUMED

An examination of column 8 shows that the fungi differed greatly in the dry weight formed from 1 gm. of sugar consumed. *Fusarium acuminatum* and *Mucor racemosus* stand out as conspicuous examples of fungi which produce a small amount of mycelium per gram of sugar used, this

being only 0.058 and 0.044 gm., respectively. Contrasted to these, *Diplodia tubericola* formed the most, namely, 0.258 gm., while *Sclerotium bataticola* and *Rhizopus tritici* formed 0.218 and 0.216 gm., respectively.

COEFFICIENT OF RESPIRATION

The number of grams of sugar reduced per gram of CO_2 evolved is shown in column 9. The differences in the case of several of the fungi are very large. *Mucor racemosus* is the most extravagant and *Botrytis cinerea* the most economical user of sugar when the comparison is made on the basis of CO_2 produced, but it may be otherwise when the dry weight of fungus substance alone is considered. Four of the fungi studied require more and three less than 1 gm. of sugar to make 1 gm. of CO_2. Kunstmann (*14*) in working with *Aspergillus niger* v. Tieg. in a 5 per cent solution of cane sugar found a considerable variation in the results obtained from different experiments. In all cases, however, more than 1 gm. of sugar was required to form 1 gm. of CO_2, the variation ranging from 1.05 to 1.98. He further showed that a greater growth of the fungus was accompanied with an increase in respiration, as would be expected, and that the respiration became more rapid as the temperature rose. He showed, furthermore, that the concentration of the cane sugar in the nutrient solution influenced the rate of respiration, it being about 1.5 times as rapid in a 30 as in a 5 per cent solution. In a solution made slightly acid with phosphoric acid (P_2O_5) the quantity of CO_2 evolved by a unit weight of growth was considerably less than in those which remained alkaline.

It is evident that there are a number of factors which might have some influence on the evolution of CO_2 in experiments with a number of fungi. It is not possible to find an environment which would be the optimum for all of them. If one considers the concentration of the solution alone with respect to the source of carbon, which Kunstmann showed to influence respiration, it is clear that a concentration which might be considered optimum for one organism might not necessarily be so for another. In spite of the great ability of fungi to adapt themselves to solutions of high osmotic concentration, it is a well-known fact that some organisms can tolerate a more concentrated solution than others. The Penicillia, for example, grow in a sugar solution of high concentration. The writers found that *Botrytis cinerea* and *Penicillium* sp. grew equally well in a concentration of 38 per cent and 58 per cent, respectively. Bearing in mind the influence the concentration has on respiration one may readily conceive how the quantity of sugar present, which in these experiments was alike for all the organisms, might work to the advantage or disadvantage of the different fungi, at least so far as the amount of sugar required to produce 1 gm. of CO_2 is concerned.

Other factors which conceivably might influence the results are (1) acidity of the solution, (2) spore sowing, (3) light, (4) temperature. As

regards the first of these, it is a well-recognized fact that most fungi prefer a slightly acid medium, and in some cases if the solution is not acid they make it so. Since this subject is to be discussed more in detail in another connection, suffice it to say here that, as with the concentration, it is probable that the degree of acidity of the original solution was not optimum for all the organisms. Kunstmann considered the question of mass inoculation of the culture by spores and found that it exercised very little influence on the rate of respiration. There is at present some difference of opinion as to the influence of light on the respiration of fungi. Kreusler (*13*) and Wehmer (*22*) deny the influence of light on growth and respiration, while Bonnier and Mangin (*1*) and Elfving (*6*) ascribe a retarding effect and Ziegenbein (*24*) a favorable influence on some flowering plants. Probably all fungi will grow in the dark. Janssens and Mertens (*10*) found that the red Torula is influenced by light and behaves like green plants, respiration being apparently more pronounced in the light than in the dark. The writers have also found that certain organisms fruit abundantly when exposed to moderately strong light but remain sterile if grown in the dark. In the writers' present experiments the fungi were exposed to light for only a short period of time each day, when the incubator was opened to adjust the apparatus or to examine the cultures.

The temperature of 29° C. at which the organisms were grown probably does not represent the optimum for many, if it does for any of these fungi. The results of many investigators have shown that there is a wide range of optimum temperatures between different organisms, some thriving as well at 37° as others at 25°. In fact, there is considerable variation between species of the same genus. Brooks and Cooley (*3*) showed that the apple-rot fungi vary in their temperature requirements, and Edson and Shapovalov (*5*) showed that certain potato-rot and wilt-producing organisms of the potato had different optimum and maximum temperatures for growth. The writers found, for example, that *Rhizopus tritici* grows better at 35° than at 29° and will make a good growth at 40° or above. Some intermediate temperature, therefore, had to be employed which would permit all the organisms to make a good growth. Furthermore, the fact must not be overlooked that the rate of CO_2 production is more or less correlated with the temperature at which the fungus is grown. Ziegenbein (*24*) found that the optimum temperature for respiration of different flowering plants varied from 35° to 45°, and Kunstmann (*14*) and Stoklasa (*19*) report a similar variation for fungi and beetroot, respectively. According to these investigators the maximum rate of respiration correlates closely with the maximum temperature for growth. When the maximum temperature for growth is reached, the rate of respiration declines rapidly.

ECONOMIC COEFFICIENT

Pfeffer (*17*) and Kunstmann (*14*) have termed the numerical relation between the sugar consumed and the dry weight of the substance formed the "economic coefficient." According to Jost (*11*) the theoretical minimum value of the coefficient is about ½, but in reality it has been found to be higher than unity. Kunstmann found in working principally with *Aspergillus niger* that it varied from 1.13 to 3.88 in parallel experiments in a 5 per cent solution of cane sugar. Ono (*16*), on the other hand, obtained values as high as 6.1.

The results obtained by the writers are shown in column 10 of Table I. It will be seen that the economic values for *Fusarium acuminatum* and *Mucor racemosus* are very much higher than any of those given by Kunstmann and Ono, being 17.11 and 22.86, respectively. The five other organisms are fairly consistent in the amount of sugar required to produce 1 gm. of dry substance, all of which, however, are equal to or higher than the maximum given by Kunstmann. In view of these facts, it is quite evident that fungi in general can in no sense be regarded as economic users of sugar. In none of the writers' experiments or in those of Kunstmann and Ono has the minimum value of the economic coefficient fallen below unity.

From the data at hand it is evident that no sweeping generalizations can be made for all fungi. Ono showed that the addition of a small amount of zinc sulphate reduced the "economic coefficient," and Jost points out that the coefficient increases with the progressive development of the fungus and with an elevation of the temperature. Since the progressive development of the fungus influences the coefficient, the element of time would have an important bearing on the results. Although all of these experiments were carried out at the same temperature (29°C.), no doubt the fungi studied did not respond to heat in a similar manner.

RESPIRATORY QUOTIENT

The dry weight of fungus material produced for each gram of CO_2 given off is in all cases considerably less than unity (Table I, column 11). To contrast the extremes, *Rhizopus tritici* formed about four times as much dry material as *Mucor racemosus*. Kunstmann in all his experiments obtained a much higher numerical value of the respiratory quotient with *Aspergillus niger* in a 5 per cent cane sugar solution. In a few cases considerably more than 1 gm. of dry weight was produced for each gram of CO_2 given off. Kunstmann's results are not in every respect comparable, since he used different temperatures in different experiments. The higher temperatures for the most part appeared to lower the value of the "respiratory quotient." However, it may be concluded that in general under experimental conditions considerably more than 1 gm. of CO_2 is given off for each gram of fungus material formed.

These variations are more or less dependent upon the temperature at which they are carried out and the length of time the experiment has run.

DISCUSSION OF RESULTS

PRODUCTS OF FERMENTATION

ALCOHOL.—It is well known that fungi often produce alcohol and various organic acids as fermentation products during respiration. If the oxidation of the sugar was complete, CO_2 and water only would be produced, but results obtained by various workers have shown that other substances are often formed. An extensive literature is extant on the production of alcohol by different fungi in culture, but no attempt will be made to review all or any considerable part of it. Suffice it to say that the results of Brefeld (*2*), Fitz (*7*), and Hansen (*8*) with different species of Mucors, Aspergillus, Rhizopus, and Penicillium, and more recenty Kostytschew (*12*) with *Aspergillus niger* show that alcohol production by fungi is not uncommon. The amount of alcohol produced by the different organisms, according to the authors cited, differs with the medium used, the temperature, and the length of time the organism was grown. *Mucor racemosus* was found by Hansen to produce as much as 7 per cent by volume in 12 months at room temperature, and Fitz showed that *M. mucedo* Bref. would form 0.8 per cent alcohol by weight in 7 weeks at a temperature of 30°C.

Obviously it is not possible to determine with any degree of accuracy by present chemical methods just what a fungus does in a solution as complex as Czapek's nutrient medium. The writers' experiments showed that alcohol was produced to a limited extent by four of the fungi studied—namely, *Fusarium acuminatum*, *Rhizopus tritici*, *Diplodia tubericola*, and *Mucor racemosus*. *R. nigricans* and *M. racemosus* were shown to be alcohol producers by other investigators, the former to a very limited extent, and the latter in considerable quantity. So far as the writers are aware, no one has reported the production of alcohol by *F. acuminatum*, *R. tritici*, or *D. tubericola*. If alcohol was produced by the other fungi it was either utilized by the fungus or formed in such a limited amount that it could not be detected by the method employed. The results from which the conclusions were drawn, although not unqualified proof, were determined by the following method: One hundred cubic centimeters of the solution were neutralized with magnesium carbonate ($MgCO_3$). Fifty cubic centimeters were then distilled off, and from this a 25-cc. fraction was taken. The iodoform test was applied to the last distillate. A positive test was obtained in most cases only upon warming. The second distillate in all cases, when the iodoform test indicated the presence of alcohol, had a lower specific gravity than water. The same tests were carried out with the control solutions with negative results.

In no case was enough CO_2 produced to account for all the glucose used up, so that the question of what became of the remainder of the glucose naturally suggested itself. Attention already has been called to the fact that many fungi produce alcohol in nutrient solutions. According to Jost (*11*) a 10 per cent solution of alcohol is usually injurious to fungi, while a 2 to 4 per cent is usually nutritive. *Sclerotium batatícola*, *Penicillium* sp., and *Botrytis cinerea* did not produce alcohol according to the iodoform test, and yet they used up all or nearly all the glucose. While the writers have no proof to offer, it is possible that alcohol was formed by these organisms which was utilized by the fungi as a source of carbon.

Positive iodoform tests were obtained for *Rhizopus tritici*, *Diplodia tubericola*, *Fusarium acuminatum*, and *Mucor racemosus*, while *Botrytis cinerea*, *Sclerotium batatícola*, and *Penicillium* sp. gave negative results. It will be seen that the last three organisms produced more than 1 gm. of CO_2 for each gram of glucose used, while the first group gave off considerably less. The distillate from the solution on which *M. racemosus* grew gave an especially heavy precipitate of iodoform crystals.

Under the conditions of the experiment *Rhizopus tritici* and *Diplodia tubericola* produced only a trace of alcohol. A separate experiment was conducted to test further the ability of the former organism to form alcohol. Flasks were prepared in triplicate, with the same medium as in the previous experiments and 10 per cent glucose as the source of carbon. All three of the flasks were inoculated, two being stoppered with rubber corks and one with a cotton plug. A normal growth of mycelium took place in the flask stoppered with cotton, but in the other two fiasks the mycelium was abnormal in appearance, less luxuriant, and mostly submerged. Distillations were made from all the solutions, and as indicated by the iodoform test an abundance of alcohol was formed in the flasks stoppered with corks, while only a mere trace could be detected in the flask plugged with cotton. It would seem then that *R. tritici* will produce alcohol much more readily when growing on the medium used in the experiments recorded above when a reduced supply of oxygen is available and intermolecular respiration is thereby induced. The concentration of the CO_2 would be increased also under these conditions. In the respiration experiment, as indicated by the amount of CO_2 produced per gram of sugar used, it is possible that considerable alcohol was formed and possibly a part of it was carried off by the air current continually passing through the flask. However, some of the sugar may have gone to form other organic substances.

ORGANIC ACIDS.—The nutrient solution with which these experiments were conducted was slightly acid at the beginning. In a paper by Weimer and Harter (*23*) it was shown that all of these same organisms when growing in a 10 per cent glucose solution increase the P_H value of the solution and some of them to a considerable extent. It has been hither-

to shown by many investigators that fungi frequently increase the acidity of the substratum. Wehmer's (*20*, *21*) work in this direction is significant. He found that *Aspergillus niger* rendered the solution at first acid. The acidity (oxalic acid) gradually increased to a maximum and declined once more during the next few weeks to zero, the solution finally becoming alkaline. The fungus was found to decompose free oxalic acid at the higher temperatures. He likewise showed that the amount of acid formed was not necessarily associated with the quantity of fungous growth produced. The acid is found only when the substratum gives no acid reaction and when the organism is cultivated in sugar, proteid, glycerin, oil, and salts of organic acids. Wehmer also found that *Citromyces glaber* Wehm. can utilize the citric acid which it has produced. Furthermore, although this organism will tolerate a concentration of 20 per cent citric acid, it only produces enough to render the substratum 4 per cent acid. In a discussion of his results he calls attention to the fact that the only acids formed by Aspergillus or Penicillium in notable quantities are oxalic and citric. *Botrytis cinerea* and *Rhizopus nigricans* and some other fungi produce oxalic acid only in traces and only in a nearly neutral medium. An abundant carbohydrate supply and calcium salts, such as calcium phosphate or carbonate, favor its production. Kunstmann (*14*) found that oxalic acid was produced in all the media used by him but that at the end of the experiment it never exceeded 0.05 gm. in 100 cc. of solution. Molliard (*15*), however, found that *Sterigmatocystis nigra* v. Tieg. produced both citric and oxalic acid, together or alone in the medium in which saccharose was used as the source of carbon. Citric acid was more abundantly produced than oxalic, but both increased gradually up to the end of the experiment. The acid production of a large number of species of Penicillium was studied by Currie and Thom (*4*), who found that it was formed in varying amounts by all of them and in a large quantity by one species in particular, *Penicillium oxalicum* Currie and Thom. According to these investigators the oxalic acid produced is not an end product. It reaches its maximum in 8 to 12 days and then declines. The results of the writers' experiments show that in no case was enough CO_2 produced to account for all the sugar consumed. Undoubtedly some of the sugar was converted into other compounds, and in this connection alcohol, aldehydes, and organic acids suggest themselves as the most likely. The presence of alcohol was demonstrated in the culture solution in which the fungus grew in the case of four organisms and not in the controls. In another paper it was shown that these same organisms growing in the solution used in these experiments increased the hydrogen-ion concentration. It seems, therefore, that alcohol or acids or both may have been produced in the solutions.

SUMMARY

(1) The following fungi can utilize glucose as a source of carbon: *Fusarium acuminatum*, *Sclerotium bataticola*, *Diplodia tubericola*, *Penicillium* sp., *Mucor racemosus*, *Botrytis cinerea*, and *Rhizopus tritici*.

(2) *Penicillium* sp., *Botrytis cinerea*, and *Sclerotium bataticola* produced a maximum of a little more than 2 gm. of CO_2 in a single day. The other fungi formed a relatively small amount. The organisms which grew rapidly produced a comparatively small amount of CO_2 and reached their maximum in a short time after the culture flasks were inoculated. In all cases the respiration was measured as long as CO_2 was given off in any measureable quantity.

(3) The three fungi, *Penicillium* sp., *Botrytis cinerea*, and *Sclerotium bataticola*, which grew slowly, produced a relatively large amount of dry material and consumed all or nearly all of the glucose. The reverse is true of the other organisms.

(4) The quantity of CO_2 evolved does not necessarily correlate with the amount of dry material formed or with the amount of glucose reduced. Some organisms (*Mucor racemosus* and *Fusarium acuminatum*) which produced a comparatively small quantity of dry material reduced a large amount of sugar.

(5) Three organisms evolved more than 1 gm. of CO_2, the others considerably less, for each gram of glucose reduced.

(6) The dry weight of material per gram oi glucose consumed is in all cases considerably less than unity.

(7) The "coefficient of respiration" varies from 0.83 to 2.01, the "economic coefficient" from 3.86 to 22.86. The "economic coefficients" of *Fusarium acuminatum* and *Mucor racemosus* (17.11 and 22,86, respectively) are several times higher than that of any of the other fungi studied. They are also higher than the values given by other investigators.

(8) The quantity of CO_2 evolved was not equivalent to the theoretical amount that might have been produced from the sugar consumed. Other investigators have shown that alcohol is formed by *Mucor racemosus*, but the writers have demonstrated for the first time that it is produced by *Fusarium acuminatum*, *Rhizopus tritici*, and *Diplodia tubericola*. It was previously shown by the writers that these same organisms when growing in a 10 per cent solution of glucose increase the acidity of the solution. It is therefore probable that some of the glucose was utilized in the production of alcohol and acids.

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HERITABLE VARIATIONS IN AN APPARENTLY UNIFORM VARIETY OF COTTON

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The Pima variety of American Egyptian cotton, which originated with a single plant selection in 1910 and of which approximately 100,000 bales were produced in 1920, is probably the least variable of commercially grown cottons. So great, indeed, is the apparent uniformity that one might question the utility of further selection within the variety and of roguing seed-increase fields which are not exposed to accidental contamination. The justification of these procedures rests upon proof that heritable variations are not infrequent, and the object of this paper is to examine the evidence of their occurrence.

It is possible to measure the results of selection in the Pima variety because, fortunately, some of the seed produced by the parent plant of 1910 had been preserved. When plants grown from this seed in 1919 were compared with the present commercial stock and with a line-bred strain it was found that although there has been no definite change of type the modes for certain characters have been altered by selection. Additional evidence of the occurrence of slight but heritable variations has been obtained in the course of the breeding and roguing work. A much wider variation, outside the normal range of the Pima variety but not attributable to recent hybridization, has also proved to be heritable.

EARLY HISTORY OF THE PIMA VARIETY

The Pima variety originated with a plant discovered at Sacaton, Ariz., in 1910, in a field of the Yuma variety. The Yuma variety likewise originated with a single plant discovered at Yuma, Ariz., in 1907 in a stock of the Mit Afifi variety which had been introduced from Egypt several years previously. The parent plants of both the Yuma and Pima varieties were so different from the stocks in which they appeared and their progenies expressed the new characters so uniformly that their origin was attributed to mutation.[2]

[1] The writer is indebted to Messrs. Walter F. Gilpin and Robert L. Taylor for the photographs used in illustrating this paper.

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No flowers on the original Pima plant were bagged for self-pollination, for its valuable qualities were not appreciated until a progeny row was grown the following year. It was then realized that a new type had arisen which differed in many characters from the Yuma variety. The progeny was remarkably uniform in view of the fact that it was grown from open-pollinated seed. The immediate progeny of the first Pima plant was grown in 1911 adjacent to a planting of the older variety, but very little hybridization could have taken place, for as far as the record shows, all plants in the five progenies which were grown in 1912 from selections made in 1911 exhibited only the distinctive Pima characters. After 1912 the Pima progenies were isolated from other cottons, but controlled self-pollination was not practiced in the earlier breeding work with this variety.[1]

A single plant selection, No. 5, in the 1911 progeny of the original Pima plant was the parent of progeny No. 5 which was grown in 1912. From a single plant selection, No. 3, in this progeny has descended the strain, henceforth referred to as 5–3, of which approximately 250,000 acres were grown in Arizona and California in 1920.

COMPARISON OF THE PRESENT COMMERCIAL STOCK WITH THE IMMEDIATE PROGENY OF THE PARENT PLANT

A progeny of 32 plants was grown in 1919 from open-pollinated seed of the original Pima plant.[2] Plates 48 and 49 show the range of variation in shape and surface of the boll, each plant of the progeny being represented, with the exception of No. 5 which was accidentally omitted.[3] Plants 1 to 17 are represented in Plate 48 and plants 18 to 32 in Plate 49. Two of these plants showed characters outside the normal range of variation of the Pima variety as it now exists. The others appeared to be typical in all respects. One of the aberrant plants (Pl. 48, L) had bolls which were conspicuously shorter, broader at the base and near the apex, and less pointed than in typical Pima, and which resembled the bolls of a first-generation hybrid of the Pima and Gila varieties. The other plant, No. 28 (Pl. 49, K), had bolls that were atypical in being more slender and less well filled out, and in having a less conspicuous "shoulder" and a more deeply pitted surface—characters which distinguish the Yuma from the Pima variety.[4] All of the 28 individuals of a progeny grown in 1920 from selfed seed of plant No. 28 produced bolls which resembled the Yuma type in greater or less degree.

Plant No. 28 of 1919 resembled the Yuma variety also in the very small amount of fuzz on the seeds, having been, in fact, the smoothest-seeded

[1] Evidence has been obtained recently that in the Egyptian type of cotton most of the ovules are self-ertilized even when no precautions are taken to prevent cross-pollination.

[2] Although the seed was nearly 9 years old, the germination was excellent and the plants made a vigorous growth.

[3] The measurements indicate that plant No. 5 had bolls which were typical in shape.

[4] Typical bolls of the Yuma, Pima, and Gila varieties of Egyptian cotton are shown on Pl. 24, fig. 2 and 3, and Pl. 25, fig. 2, *in* KEARNEY, Thomas H., OP. CIT. 1914.

of the 32 plants of the Pima parent progeny.[1] The progeny grown in 1920 showed considerable variation in this character, but a majority of the plants had smoother seeds than the average Pima and a few individuals had almost naked seeds. It is therefore probable that plant No. 28 was a first-generation Pima × Yuma hybrid, the original Pima plant of 1910 having been surrounded by plants of the Yuma variety. It is surprising, under the circumstances, that in a progeny of 32 individuals only 1 showed recognizable Yuma characters.[2]

In order to measure the degree of change which might have taken place as a result of several years of selection in the Pima variety, the progeny of the parent plant grown in 1919 was compared with an equal

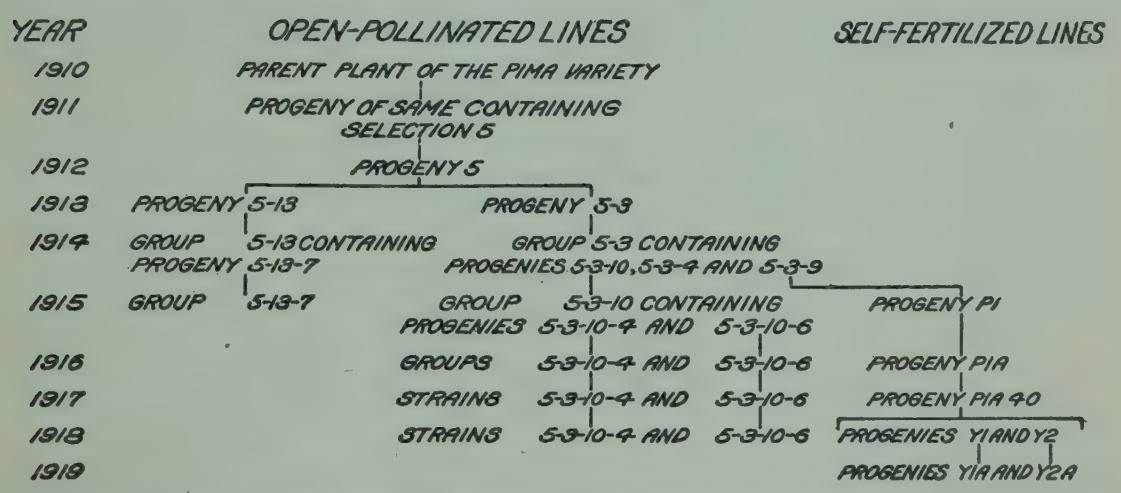


FIG. 1.—Pedigree of progenies, progeny groups, and strains of the Pima variety of American Egyptian cotton. Each progeny is from an individual plant selection of the preceding year. As here used, the term "progeny group" comprises the progenies of sister plants of the preceding year and the term "strain" comprises all lines derived from the same progeny group.

number of plants taken at random in the commercial stock of strain 5–3 and of two progenies (Y1A and Y2A) derived from the same strain by five generations of controlled self-fertilization. The ancestry of these selfed lines is indicated in figure 1. For the sake of brevity, the three populations will be referred to hereafter as "parent progeny," "5–3 bulk," and "5–3 selfed." All were grown under conditions of soil, irrigation, and spacing which were as nearly as practicable identical.

[1] The apparent dominance of a less fuzzy over a more fuzzy condition of the seed coat in this presumable first-generation hybrid is exceptional, since according to Balls (BALLS, W. Lawrence. SOME CYTOLOGICAL ASPECTS OF COTTON-BREEDING. *In* Amer. Breeders' Assoc. Rpt. 1908, v. 5, p. 18. 1909.) "little fuzz" is dominant over "no fuzz" and "entire fuzz" is dominant over "little or no fuzz". Similar relations are reported by McLendon (MCLENDON, C. A. MENDELIAN INHERITANCE IN COTTON HYBRIDS. Ga. Agr. Exp. Sta. Bul. 99, p. 169. 1912.). It has been the writer's experience that in hybrids between Egyptian varieties with partly fuzzy seeds and Upland varieties with completely fuzzy seeds the first generation has completely fuzzy seeds.

[2] Progenies comprising from 22 to 34 individuals were grown in 1920 from selfed seed of 5 plants of the "parent" progeny of 1919, and the leaf and boll characters listed in Table I were determined for all plants of each progeny. Comparison of the means of these progenies with the means of selfed and open-pollinated stocks of strain 5–3 showed no important differences, except that the mean boll diameter and boll index (diameter as a percentage of the length) of the progeny of plant 28 were significantly smaller than in any other progeny or strain of Pima on which these characters were measured in 1920. Comparison of the coefficients of variation for the several characters showed that progeny 28 was decidedly more variable than the other granddaughter progenies of the parent Pima plant, as would be expected if this progeny represented the second generation of a Pima × Yuma cross.

Measurements were made on one leaf and one boll of each plant,[1] endeavor having been made to select in each case a leaf and a boll which had developed normally and which appeared to be typical of the plant in question. Seed cotton was collected from 5 open-pollinated bolls on each plant, and the fiber on one seed from each boll was combed out and measured, the average of the 5 determinations being taken as the length of fiber for the plant in question. The seed cotton was then weighed and ginned, the weight of the fiber and the number of seeds in the sample were determined, and the lint index (weight in grams of fiber per 100 seeds) was calculated from the resulting data. Finally, the seeds were graded in respect to fuzziness by matching with a set of types representing the total range from the smoothest (grade 1) to the fuzziest (grade 7) condition of the seed coat. Table I shows for the 12 characters the means and coefficients of variation of the three stocks.

TABLE I.—*Means and coefficients of variation for 12 characters of a progeny of the parent plant of the Pima variety, of a "bulk" (continuously open-pollinated) population of strain 5–3, and of a line of the same strain which had been strictly self-fertilized during five generations*

Character.	Means.			Coefficients of variation.[1]		
	Parent.	Bulk.	Selfed.	Parent.	Bulk.	Selfed.
Leaf length (cm.)[2]	169±1.14	164±2.27	167±1.41	5.4±0.47	11.1±0.96	6.8±0.59
Leaf width (cm.)[3]	219±2.71	214±3.53	229±2.09	9.9± .86	13.2±1.15	7.3± .64
Leaf index [4]	129±1.37	130±1.35	137±1.11	8.5± .74	8.3± .72	6.5± .56
Leaf vein angle [5]	106±1.37	104±1.42	114±1.15	10.3± .90	11.0± .96	8.1± .70
Leaf lobe index [6]	268±2.06	261±1.64	284±2.38	6.1± .53	5.0± .44	6.8± .59
Leaf extension index [7]	19.5± .37	17.1± .46	20.5± .40	15.2±1.32	21.5±1.87	15.5±1.35
Boll length (mm.)	46.2± .24	47.1± .21	47.0± .23	4.4± .40	3.7± .31	4.1± .35
Boll diameter (mm.)[8]	27.3± .14	26.9± .08	27.9± .07	4.2± .35	2.6± .22	2.1± .18
Boll index [9]	59.2± .33	57.3± .28	59.5± .33	4.6± .39	4.0± .34	4.6± .39
Fiber length (mm.)	40.8± .19	44.6± .12	42.9± .12	3.8± .32	2.2± .19	2.4± .20
Lint index (gm.)	4.1± .06	5.6± .05	4.5± .06	13.2±1.11	7.9± .67	11.1± .94
Seed fuzziness	3.8± .17	4.1± .06	4.7± .11	1.4± .12	.5± .04	.9± .08

[1] In the case of seed fuzziness (graded) the standard deviations are compared. For all other characters the comparison is of coefficients of variation.
[2] Measured from base of midvein to tip of central lobe.
[3] Measured from tip to tip of the principal lateral lobes.
[4] Width as a percentage of the length, instead of length as a percentage of the width, the value used in the paper by Kearney and Wells, cited on p. 227. The new leaf index is the reciprocal of the old, multiplied by 10,000.
[5] Width (in degrees) of the angle formed by the principal lateral veins.
[6] Leaf length as a percentage of the distance from the base of the midvein to the bottom of the upper right hand sinus. A deeply lobed leaf has a high lobe index and vice versa.
[7] Length of the basal extension of the blade, below a line bisecting the base of the midvein at a right angle to the latter, as a percentage of the length of the leaf.
[8] At the point of greatest diameter.
[9] Maximum diameter as a percentage of the length, instead of length as a percentage of the diameter, the value used in the paper by Kearney ahd Wells, cited on p. 227. The new boll index is the reciprocal of the old, multiplied by 10,000.

The coefficients of variation of the parent progeny, as compared with those of the 5–3 bulk, were higher in 8 and lower in 4 of the 12 characters;

[1] Since only 30 plants of the "Pima parent" progeny had leaves in good condition for measurement, the leaf characters were determined on only 30 plants in the other two stocks. The boll, fiber, and seed characters were determind on all 32 plants of each stock. The leaf characters were measured on selfed progeny Y1A and the other characters on selfed progeny Y2A.

and as compared with the selfed line they were higher in 8 and lower in 3 characters. If we regard as significant only such differences as amount to at least three times the probable error, the parent progeny was significantly more variable than the bulk stock in 4 and significantly less variable in 1 of the 12 characters. As compared with the selfed population, the parent progeny was significantly more variable in 3 and significantly less variable in none of the characters.

The high degree of homozygosity apparently attained by the commercial stock as a result of simple selection without controlled selfing is indicated by the fact that five generations of controlled selfing has rendered the "selfed" line significantly less variable than the "bulk" stock in only 2 of the 12 characters, while in 1 character it is significantly more variable. No significant difference in variability is shown in respect to the 9 remaining characters.

FIBER AND SEED CHARACTERS OF THE PARENT PROGENY AND OF THE PRESENT COMMERCIAL STOCK

Especial interest attaches to the characters fiber length, lint index, and seed fuzziness because of their practical importance and because since the commencement of the breeding work with the Pima variety attention has been focused upon increasing the length and abundance of the fiber and obtaining a high degree of uniformity in these characters. An indication of what has been accomplished along these lines is afforded by the frequency distributions in Table II. The maximum and minimum length of fiber, abundance of fiber, and fuzziness of seeds of the parent progeny and of a corresponding number of plants of the commercial stock are represented in Plates 50, 51, and 52.

LENGTH OF FIBER.—It is evident from the data in Tables I and II that the immediate progeny of the parent plant of the Pima variety was much more variable in length of fiber and had a significantly lower mean for this character than a random sample of the present commercial stock of strain 5–3. This strain, as indicated in the pedigree shown in figure 1, was derived from a granddaughter of the original Pima plant, selected in 1912 largely on the basis of its superior length of fiber. The difference between the means of the "parent" progeny and of the bulk stock was 3.8 mm., hence more than ⅛ inch, and the difference was 17 times greater than its probable error. The selfed line of strain 5–3 was almost intermediate in length of fiber between the "parent progeny" and the "bulk," its mean differing from those of the other two populations by amounts equivalent to 9 and 10 times the probable error of the differences. The ancestors of this closely inbred line, beginning with plant P1 in 1914, had not been selected with reference to this character and would seem to have represented very nearly the minimum potentiality of strain 5–3 in respect to length of fiber. The coefficients of variation of the bulk and selfed populations do not differ significantly, but both were significantly less variable than the parent progeny.

TABLE II.—*Frequency distributions for the characters fiber length, lint index, and seed fuzziness of a progeny of the parent plant of the Pima variety, of a "bulk" (continuously open-pollinated) population of strain 5-3, and of a line of the same strain which was strictly self-fertilized during five generations, each population containing 32 plants*

FIBER LENGTH

	37 mm.	38 mm.	39 mm.	40 mm.	41 mm.	42 mm.	43 mm.	44 mm.	45 mm.	46 mm.	47 mm.
Parent progeny	1	3	2	6	11	5	1	2	1	....	....
5–3 bulk	....	....	....	....	....	....	5	9	13	4	1
5–3 selfed	....	....	....	2	2	4	15	6	3	....	....

LINT INDEX

	3.3 gm.	3.8 gm.	4.3 gm.	4.8 gm.	5.3 gm.	5.8 gm.	6.3 gm.	6.8 gm.	7.3 gm.
Parent progeny	4	10	13	2	3	.....	.....	.....	.....
5–3 bulk	.....	.....	.....	1	18	10	2	.....	1
5–3 selfed	.....	4	14	10	3	.....	1	.....	.....

SEED FUZZINESS

	Grade 1.	Grade 2.	Grade 3.	Grade 4.	Grade 5.	Grade 6.	Grade 7.
Parent progeny	1	5	7	8	8	3	.....
5–3 bulk	.....	.....	3	23	5	1	.....
5–3 selfed	.....	.....	1	13	13	3	2

QUALITY OF FIBER.—While no quantitative data on this point are available, it seemed evident in examining the samples of fiber from the individual plants of the three stocks that those of the "parent" progeny differed more among themselves in smoothness, luster, and color than did the plants of the "bulk" stock and of the selfed line. The smoothness or silkiness of the fiber also seemed to average higher in the two latter populations. Selection, therefore, would seem to have resulted in raising the mean of this character and in reducing its variability.

LINT INDEX.—The abundance of the fiber, as expressed by the mean weight of fiber borne by 100 seeds, averaged 36½ per cent greater in the "bulk," or commercial stock of the variety, than in the progeny of the original Pima plant. The difference between the means is very significant, amounting to 19 times its probable error. There had been also a significant reduction in variability, the coefficient of variation for lint index of the "bulk" stock being lower than that of the "parent" progeny by an amount equivalent to more than 4 times the probable error of the difference. Since increase in the abundance of fiber on the seed, like increase in length of fiber, has been one of the principal objectives of the breeding work with the Pima variety, it is interesting to

note this evidence of the progress attained. On the other hand, the selfed line, the immediate ancestors of which had not been selected with reference to this character, had a much lower mean and a higher, though perhaps not significantly higher, coefficient of variation for lint index than the bulk population of the 5–3 strain from which it was derived. In this character, also, five generations of controlled self-fertilization apparently had resulted in no further progress toward homozygosity.

FUZZINESS OF SEEDS.—In the earlier years of breeding work with the Pima variety no especial attention was given to this character. Consequently it is not surprising that the "bulk" stock, as compared with the parent progeny, showed no significant difference in the mean amount of fuzz on the seed. On the other hand, the variability had been significantly reduced, the standard deviation of the bulk stock being lower than that of the parent progeny by an amount equal to 7 times the probable error of the difference. The selfed line had a mean fuzziness which was significantly greater than that of both the "bulk" stock and the parent progeny, the difference in both cases being about five times its probable error. The selfed line was significantly more variable in seed fuzziness than the bulk stock but was significantly less variable than the parent progeny. It is evident that the selfed line had been unconsciously selected towards the upper limit of fuzziness represented in the immediate progeny of the parent plant of the Pima variety. Fuzziness is a marked disadvantage from a practical point of view, the separation of the fiber by the type of gin used for this very long staple cotton being much more easily effected with smooth than with fuzzy seeds. The frequency distribution of the parent progeny indicates that selection of a strain having smoother seeds than the present commercial stock of Pima is well within the limits of possibility.[1]

EVIDENCE OF THE OCCURRENCE OF HERITABLE VARIATIONS OBTAINED IN ROGUING

The Yuma variety of American Egyptian cotton was grown by Arizona farmers previous to the introduction of the Pima variety. In 1914, 1915, and 1916 fields of this cotton were rogued by agents of the United States Department of Agriculture, in cooperation with the Salt River Valley Egyptian Cotton Growers' Association, in order to supply seed to be increased for planting the commercial acreage. The total number of plants in the fields rogued during the three years was rather closely estimated as 2,290,000, of which (by actual count) 23,537, or 1.03 per cent, were removed as being off-type or otherwise undesirable. The work involved a rapid examination of every plant in the area rogued and thus afforded an excellent opportunity for judging the state of uniformity of the variety.

[1] This is true even if we leave out of consideration the single plant (No. 28) which falls into grade 1, this having been, in all probability, a first-generation Pima × Yuma hybrid.

A more careful examination was made at Sacaton in 1914 of 124 individuals taken at random in a field planted with ordinary "gin run" seed of Yuma cotton. Of these, 117 were classified as true Yuma, while 7, or 5.5 per cent of the total, were classified as distinctly off-type. Progenies grown in 1916 from selfed seed of the off-types and of plants regarded as typical Yuma proved to be, in most cases, remarkably uniform in the stem, leaf, and boll characters which had distinguished the parent individuals. A form which appeared to be analogous to the so-called "bull-stalk" variation in Sea Island cotton, characterized by a tall stem, long internodes of the axis and fruiting branches and rather stiff, semi-erect leaves, was so frequent as to be included within the normal range of variation of Yuma cotton, although this type also proved to be heritable.

Cooperative roguing was carried on during and after the substitution of the Pima for the Yuma variety. In 1916, 1917, 1919, and 1920 an estimated total of 3,600,000 plants were examined; and, by actual count, 10,221 plants, or 0.28 per cent of the total number, were removed from the seed-increase fields of the Pima variety. There was little variation in the amount of elimination from year to year, the figures having been 0.21 per cent in 1916, 0.42 per cent in 1917, 0.20 per cent in 1919, and 0.20 per cent in 1920. Although the roguing was much more rigorous than that practiced with the Yuma variety, the percentage of the total plants which were removed from the Pima fields in all four years was less than one-third as great. Most of the Pima "rogues" departed so little from the type that they would have been disregarded in roguing a field of the much more variable Yuma. A large majority of the plants removed were merely sickly or more or less sterile. Indisputable evidence was therefore obtained that the closer line-breeding which had been practiced from the beginning with the Pima variety had resulted in the development of a much more uniform stock.

The most distinct off-types in the Pima fields, in addition to a few very aberrant individuals of which the characters indicated hybridization with some other variety of cotton, were either tall, vigorous individuals having exceptionally long internodes (the "bull-stalk" type) or stiff, slender, grayish plants having semierect branches and leaves, the leaves being narrower, more deeply lobed, and with more incurved and undulate margins than in typical Pima. Both of these forms had their counterparts in the older Yuma variety, and it seems not improbable that they arise by recurrent mutation or recombination in all varieties of Egyptian cotton. Variation in the shape of the bolls was much less frequent and extreme than in the Yuma variety, but a few of the plants had shorter and less pointed, or longer and more taper pointed, bolls than in typical Pima.

In a bulk field of Pima cotton at Sacaton in 1917 certain individuals were selected as slightly off-type. Flowers were bagged on these plants,

and progenies were grown in 1919 from the resulting selfed seed. One of these progenies resembled the parent individual in having the surface of the boll more deeply pitted than in typical Pima. Another progeny showed a tendency to produce shorter and plumper bolls than the Pima type, while a third progeny showed the "bull-stalk" habit as well as a tendency to produce short, plump bolls. So far as this evidence goes it indicates the inheritance of slight variations in the Pima variety.

The recognition of these various types is always easiest in a well-grown field of cotton. If the plants are either too rank or too stunted, the variations are more or less masked. Certain soil conditions appear to favor the expression of a certain type. Thus, fields have been examined where the growth was subnormal and where all the plants showed a tendency to the stiff, grayish form which in most fields occurs scatteringly among plants of normal habit and color. On the other hand, where the growth is very rank, the "bull-stalk" type can scarcely be recognized.

EVIDENCE OF THE OCCURRENCE OF HERITABLE VARIATIONS OBTAINED IN LINE BREEDING

The records of the breeding work (selection) which has been carried on with the Pima variety since its origin in 1910 have been scanned for evidence of the occurrence of heritable variations. Unfortunately the stem, leaf, and boll characters had not been recorded systematically, but a few data based upon careful measurements are available. The type of plant which has been kept in mind as the ideal in breeding work with the Pima variety is shown in Plate 53. It is characterized by the absence of vegetative branches and by the development of good fruiting branches from near the base to the summit of the axis. The relationship of the various strains and progenies mentioned in the following pages is shown in figure 1.

In 1914 two groups (5–3 and 5–13), descended from sister plants in progeny No. 5 of 1912, were compared with respect to certain stem and branch characters and showed significant differences, as given in Table III.

TABLE III.—*Means for stem and branch characters in two lines of the Pima variety grown in 1914*

Character.	Group 5–3 (24 plants).	Group 5–13 (26 plants).	Differences between means.
Axis length (cm.)	178. 0± 1. 55	194. 5± 2. 11	16. 5± 2. 62
Axis internode number	40. 5± .34	42. 8± .43	2. 3± .55
Axis internode length (cm.)[1]	44. 0± .33	45. 5± .25	1. 5± .41
Limb index [2]	102. 3± 10. 76	172. 4± 12. 88	70. 1± 16. 80

[1] Value for each plant obtained by dividing the length of the axis by the number of internodes.
[2] Aggregate length of vegetative branches divided by length of axis.

In 1915 the two strains, as represented by progeny groups 5–3–10 and 5–13–7, were further compared in regard to length of axis and development of the vegetative branches, with the results given in Table IV.

TABLE IV.—*Means for axis length and limb index in two lines of the Pima variety grown in 1915*

Character.	Group 5–3–10 (25 plants).	Group 5–13–7 (15 plants).	Differences between means.
Axis length (cm.)	148± 1.28	176± 2.96	28± 3.23
Limb index	115±12.30	144±12.56	29±17.60

The differences in the means for axis length and limb index are in the same direction as those for strains 5–3 and 5–13 in 1914, but the difference in limb index in 1915 was less than twice its probable error. This character, however, is extremely sensitive to environmental influence, and it is a fair assumption that the two strains are genetically different in respect to both the length of the axis and the development of the vegetative branches.

The records for 1914 permit comparison of still more closely related lines. In that year progenies were grown of several plants which had been selected in progeny 5–3 of 1913. One of these progenies (5–3–4) was noted as being very uniform and very distinct from the others, the plants having been stiff and open looking, with long fruiting branches, long internodes of the axis and fruiting branches, and rather stiff leaves which were deeply and narrowly lobed. This description recalls the "bull-stalk" type which was encountered frequently in roguing the seed-increase fields, except that the plants were not noticeably taller than in the other progenies. The differences in stem and branch characters, as compared with the progeny of a sister plant (5–3–10), are shown by the data in Table V.

TABLE V.—*Means for stem and branch characters of two progenies from sister plants, grown in 1914*

Character.	Progeny 5–3–4 (12 plants).	Progeny 5–3–10 (11 plants).	Differences in the means.
Axis length (cm.)	183.0±1.80	179.0±2.19	4.0±2.83
Axis internode number	38.0± .25	41.2± .39	3.2± .46
Axis internode length (cm.)	48.2± .35	43.5± .66	4.7± .75
Fruiting branch length (cm.)	63.2±1.19	56.5± .78	6.7±1.42

Progeny 5–3–4 had significantly fewer and significantly longer internodes of the axis and significantly longer fruiting branches than progeny 5–3–10, the differences having been, respectively, 6.9, 6.3, and 4.7 times their probable errors.

Two plants, selected in progeny 5–3–10 of 1914 and designated 5–3–10–4 and 5–3–10–6, gave rise to strains of which progenies were grown in 1916, 1917, and 1918. Measurements of the weight of seed cotton per boll, length of fiber, lint index (weight of fiber per 100 seeds), and fuzziness of the seeds were made in each year upon plants of each strain. Consistent and significant differences were found only in regard to the character seed fuzziness. The means of the grade numbers of the individual plants are given in Table VI.

TABLE VI.—*Means for seed fuzziness of two closely related lines of the Pima variety, grown in 1916, 1917, and 1918*

Group or strain.	1916		1917		1918	
	Number of plants.	Mean grade.	Number of plants.	Mean grade.	Number of plants.	Mean grade.
5–3–10–4	16	3.9±0.11	47	3.2±0.17	71	3.3±0.10
5–3–10–6	29	4.4± .11	35	3.8± .15	28	4.3± .10
Difference	.....	.5± .155	.....	.6± .226	.....	1.0± .142

Strain 5–3–10–6 is consistent in having had fuzzier seeds in each of the three years, although the difference in 1917 was scarcely significant. This case affords clear evidence of a slight but heritable difference between two lines which are very closely related, and of which the common ancestor (plant 5–3–10 of 1913) represented the third generation of linebred descent from the parent plant of the Pima variety.

INHERITANCE OF AN INCREASED NUMBER OF BOLL LOCKS

The boll of Pima cotton has usually 3 locks or carpels, although 4-lock bolls are borne by nearly all plants of this variety and bolls having 2 or 5 locks are met with occasionally. There is much individual variation in the proportion of 3-lock and 4-lock bolls. With a view to ascertaining whether such differences are heritable, several plants which had more or fewer 4-lock bolls than the average were selected in 1917 in an increase plot of a selected strain of Pima. Progenies of three such plants were grown in 1918 from seed produced by open-pollinated flowers. Table VII gives the percentages of bolls with 4 locks, all bolls on all plants in each progeny having been taken as one array in computing the percentage and its probable error.

TABLE VII.—*Percentages of 4-lock bolls in the progenies of individual plants selected in 1917*

Progeny.	Number of plants.	Percentage of 4-lock bolls.
No. 2 (from parent with few 4-lock bolls)	19	4.6±.031
No. 1 (from parent with many 4-lock bolls)	22	5.9±.33
No. 3 (from parent with many 4-lock bolls)	8	10.5±.67

The difference as between progenies 2 and 1 was barely three times its probable error, while as between progenies 2 and 3 it was more than six times its probable error.

Progenies were grown in 1919 from selfed seed of one plant in progeny No. 3 and of two plants in progeny No. 1 of 1918. In two of the progenies of 1919, flowers on a few plants were selfed and progenies from the resulting seed were grown in 1920. The percentages of 4-lock bolls on the plants of these progenies and on plants taken at random in a neighboring plot of bulk Pima, in both years, are given in Table VIII.

TABLE VIII.—*Percentages of 4-lock bolls in 1919 and 1920 in the progenies of plants selected for a high percentage of such bolls and in "bulk" plantings of Pima cotton*

Material.	1919		Material.	1920	
	Number of plants.	Percentage of 4-lock bolls.		Number of plants.	Percentage of 4-lock bolls.
4-lock progenies:			4-lock progenies:		
1–3	13	8.5±0.65	1–3–12	45	9.2±0.36
1–5	20	8.7± .54	3–2–4	39	22.0± .62
3–2	5	10.9± .87	3–2–5	42	23.3± .60
Combined	38	9.1± .37	Combined	126	17.2± .30
Bulk Pima	38	4.2± .27	Bulk Pima	50	5.3± .29

The descendants of plants which had been selected as showing a tendency to increased number of locks gave, in both years, significantly higher percentages of 4-lock bolls than plants taken at random in the general stock of the Pima variety. In 1919 the difference in favor of the 4-lock progenies was 10 ½ times and in 1920 it was 28 ½ times its probable error. In all three years (1918 to 1920) the descendants of selection No. 3 of 1917 gave a higher percentage of 4-lock bolls than the descendants of selection No. 1. These facts indicate clearly the heritable nature of this variation.

There can be little doubt, therefore, that a strain having a materially higher percentage of 4-lock bolls could be developed by selection, although the practical importance of such increase in the Pima variety would seem to be small. Determination by Mr. W. G. Wells in 1919 of the mean weight of seed cotton per boll in 100 3-lock and 100 4-lock bolls showed that the latter averaged only 10 per cent heavier than the former. Similar determinations by Mr. W. F. Gilpin in 1920 on 50 bolls of each lock number showed a difference of only 7 per cent in favor of the 4-lock bolls. Hence, even if it were possible to develop a strain of Pima cotton in which all the bolls would have 4 locks, the increase in yield of fiber and seed would not be likely to exceed 10 per cent.

INHERITANCE OF SPOTLESS OR FAINTLY SPOTTED PETALS

The variations which have been considered thus far are so slight that in the absence of evidence of their heritability, they would be regarded as mere fluctuations. Especial interest attaches to a variation which is outside the normal range of variation not only of the Pima variety but of the Egyptian type of cotton.

Inspection of the 4-lock progenies in 1919 revealed the surprising fact that in all plants in these three progenies there was either total absence or very faint development of the dark red spot near the base of the petal which is so marked a characteristic of the Pima variety and of Egyptian cotton in general. In this respect the 4-lock plants contrasted strikingly with all other Pima progenies in the same field as well as with the bulk stock of this variety grown in adjacent fields. Although there is appreciable variation in the size and in the intensity of color of the petal spot in Pima cotton, no such approach to complete absence of the spot had been observed hitherto except in plants which were obviously hybrids with Upland cotton.

The 38 plants of the three 4-lock progenies in 1919 were graded by Mr. W. G. Wells with respect to degree of development of the petal spot, grade 8 indicating the extreme development in typical Pima cotton and grade 0 indicating complete absence of the spot, as is the case in most varieties of American Upland cotton (*Gossypium hirsutum L.*). Two or three flowers were separately graded on each plant, and the averages of the resulting grade numbers were used in computing the mean for the entire population, which was 1 ± 0.04 as compared with a mean of 7.5 ± 0.19 for a Pima population of 13 plants which had normally developed petal spots. Of a total of 106 flowers examined, in the 4-lock progenies, 16 on 14 different plants showed no trace of a petal spot, and none of the flowers was graded higher than 2.

Four progenies, descended from two of the individuals which were selected in 1917 [1] for a high percentage of 4-lock bolls, were grown in 1920. Ten flowers on each plant in these four progenies were graded by the writer with respect to the degree of development of the petal spot, and the progeny means were computed from the mean grade of the 10 flowers per plant. The results are stated in Table IX.

TABLE IX.—*Mean grade of petal spot development in the 4-lock progenies of 1920*

Progeny.	Number of plants.	Number of flowers.	Mean grade of petal spot.
1–3–2	15	150	1.0±0.06
1–3–12	45	450	1.0± .04
3–2–4	38	380	.9± .04
3–2–5	42	420	.9± .03

[1] It is a remarkable coincidence that of two individuals which were selected as having a high percentage of 4-lock bolls, although at the time of selection this peculiar character of the flowers was not noted, the descendants have shown themselves to be alike in the subnormal development of the petal spot. As it is unlikely that this character would have been overlooked if it had been common to all plants of the strain in which these selections were made in 1917, the most probable explanation of the coincidence is that the two individuals in question were sisters.

Of the 1,400 flowers which were examined in these four progenies, 459, or 33 per cent, showed no trace of a petal spot, and 165, or 12 per cent, showed only the faintest discernible trace. Only 27 flowers were graded No. 3, and only 4 flowers were graded No. 4, the highest grade which was represented. None of the plants showed total absence of the spot in all 10 of the flowers which were examined, but on 1 plant each in progenies 1–3–2 and 3–2–4, 9 of the flowers showed no trace of the spot and the tenth flower showed only a faint trace (grade 1).

The range of variation in petal spot development shown by the great majority of the 4-lock plants in 1920 (from 0 to grade 2) as compared with the normal development in Pima, the total absence of the spot in the Holdon variety of Upland cotton, and a somewhat subnormal development in the first generation of a hybrid between Pima and Holdon, are illustrated in Plate 54.

The strongly hereditary nature of this variation is shown by the absence or slight development of the spot on all plants of the three progenies in 1919 and of the four progenies in 1920, whereas in both years all other stocks of the Pima variety which were growing in the same field and on which observation of this character was made showed a normal development of the petal spot.

In regard to other color characters of the flowers, the petals on every plant in the 4-lock progenies in 1919 and 1920 had the full lemon-yellow color and the anthers had the full orange-yellow color which is characteristic of Pima, and in all other characters, excepting the greater number of 4-lock bolls and the absence or very slight development of the petal spot, no appreciable departure from the type of the variety was observed.

The tendencies to increased lock number and to disappearance of the petal spot in these lines suggest that there may have been non-Egyptian "blood" in their remote ancestry. In most varieties of American Upland cotton, as well as in the Hindi or "Weed cotton" of Egypt,[1] 4-lock bolls predominate and the petal spot is absent. There had been no opportunity for hybridization with either of these types subsequent to the origin of the Pima variety, but the possibility is not excluded that the factors in question were introduced through the Yuma ancestors of the Pima parent, the Yuma variety having perhaps received them from its Mit Afifi progenitors.

This association of very weak development or total absence of the petal spot with a relatively high percentage of 4-lock bolls suggests the existence of a negative correlation between petal spot and mean lock number. Within the 4-lock populations, however, no significant correlation could be detected; and in the second generation of a hybrid between Egyptian and Upland cottons, of which 180 plants were grown at Sacaton in 1919, this pair of characters proved to be uncorrelated, the

[1] COOK, O. F. HINDI COTTON IN EGYPT. U. S. Dept. Agr. Bur. Plant Indus. Bul. 210, 58 p., 6 pl. 1911.

coefficient of correlation having been 0.01 ± 0.05. The means of 22 progenies of the third generation of this hybrid, grown in 1920, showed a positive rather than a negative but not a significant correlation, the coefficient having been 0.22 ± 0.14. The absence of a significant and negative correlation between these characters in the second and third generations of a known hybrid between Egyptian and Upland cottons would be evidence, if evidence were needed, that the association of a high percentage of 4-lock bolls with great reduction of the petal spot in these Pima lines is not attributable to recent hybridization with Upland cotton.

SUMMARY

Evidence is presented in this paper of the occurrence of heritable variations in the Pima variety of American Egyptian cotton, which is probably the most uniform variety of cotton now grown on an extensive scale.

Comparison of a progeny grown from seed of the parent individual of the variety with the present commercial stock proves that there has been significant improvement in the length and abundance of the fiber, as well as in the uniformity of these characters. This is shown by comparison of the means and coefficients of variation (Table I) and of the frequency distributions (Table II).

Indications of the occurrence of heritable variations have been obtained in roguing fields of the Pima variety, although the variations are much less numerous and are of much smaller magnitude than those which were observed in fields of the older Yuma variety.

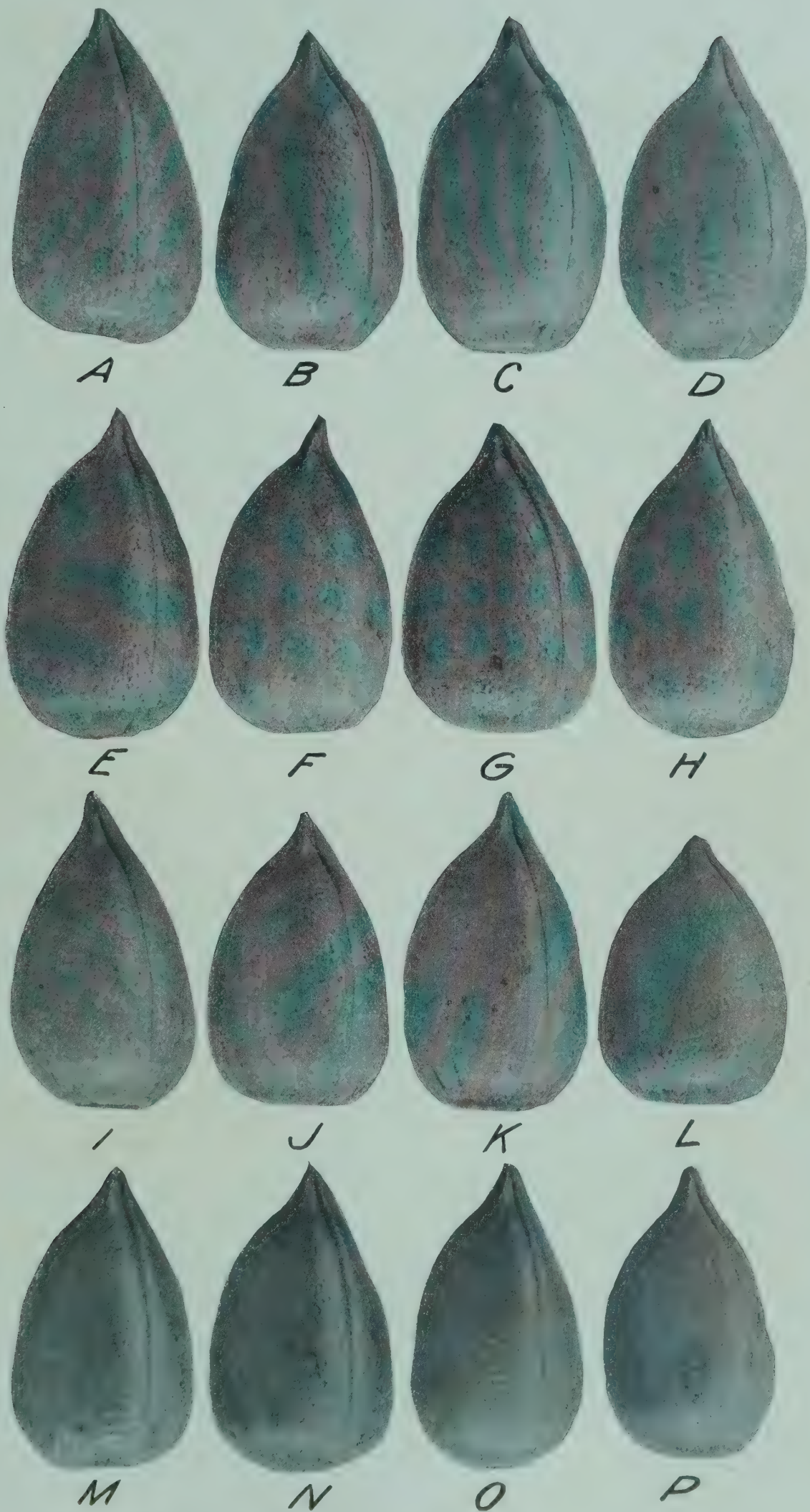
The records of the breeding work with Pima cotton supply additional evidence of the occurrence of slight, heritable variations, none of which could be considered as outside the normal range of variation of this variety. They indicate, however, that something may be accomplished by selection in regard to characters of practical importance.

A much more striking variation, characterized by the complete or nearly complete absence of the dark red spot near the base of the petal, associated with an increased percentage of 4-lock bolls, was found to be heritable in a high degree. The nature of this variation and the circumstances of its occurrence suggest the possibility that Upland cotton (*Gossypium hirsutum*) or Hindi cotton (*G. punctatum* Sch. and Thon.?) may have been involved in the remote ancestry of the Pima variety.

The fact that heritable variations are found in this apparently uniform variety is thought to justify the continuance of selection and line breeding and the roguing of seed increase fields.

PLATE 48

A progeny of 32 plants was grown in 1919 from seed of the original Pima plant of 1910, and an opportunity was thus afforded for determining the result of selection since the origin of the variety. Representative bolls, one from each plant of this "parent progeny" are shown in this and the following plate, plants 1 to 17 being shown in Plate 48 and plants 18 to 32 in Plate 49. All are within the normal range of the variety as it now exists, with the exception of Plate 48, L, and Plate 49, K. Slightly reduced.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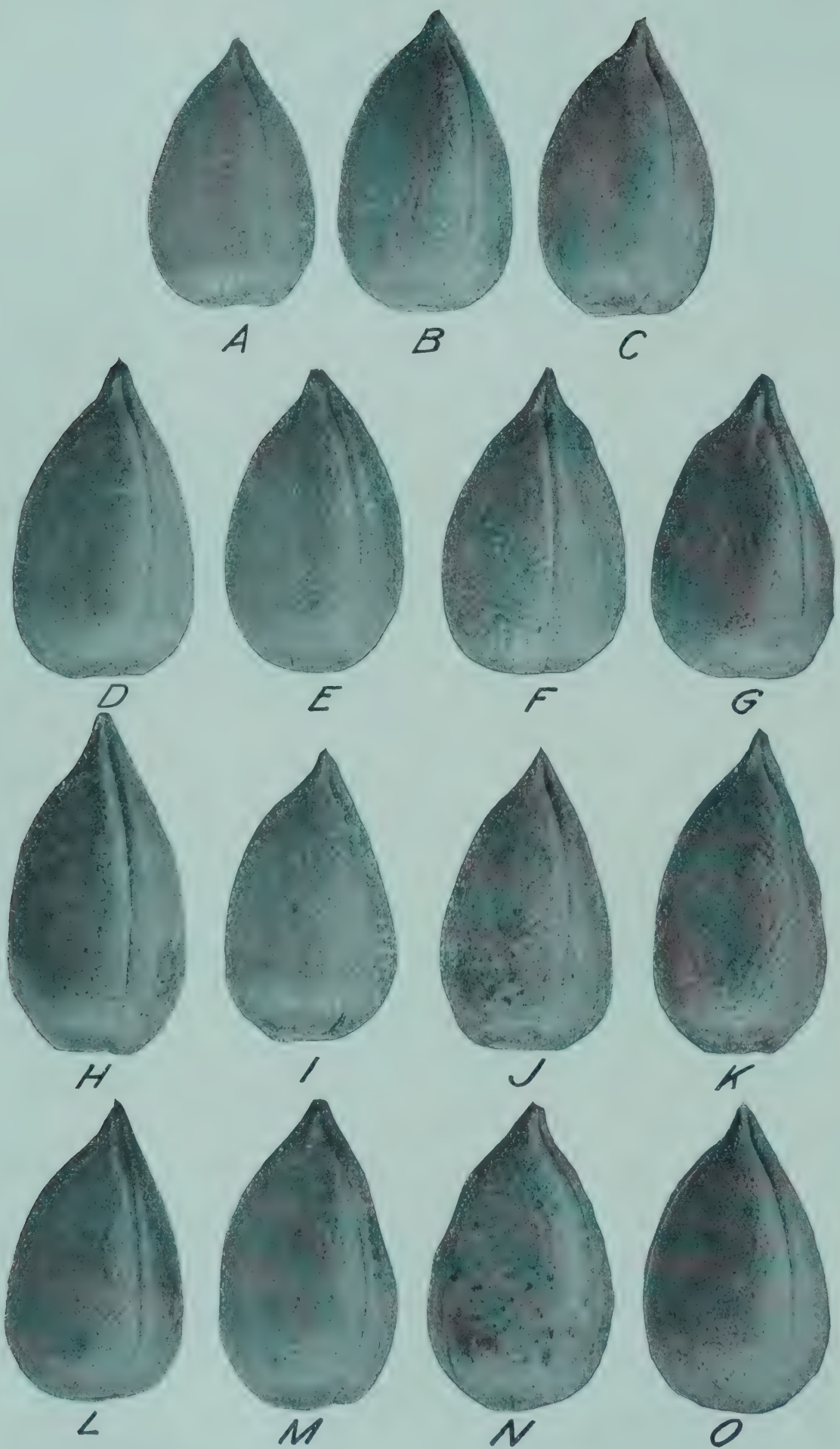
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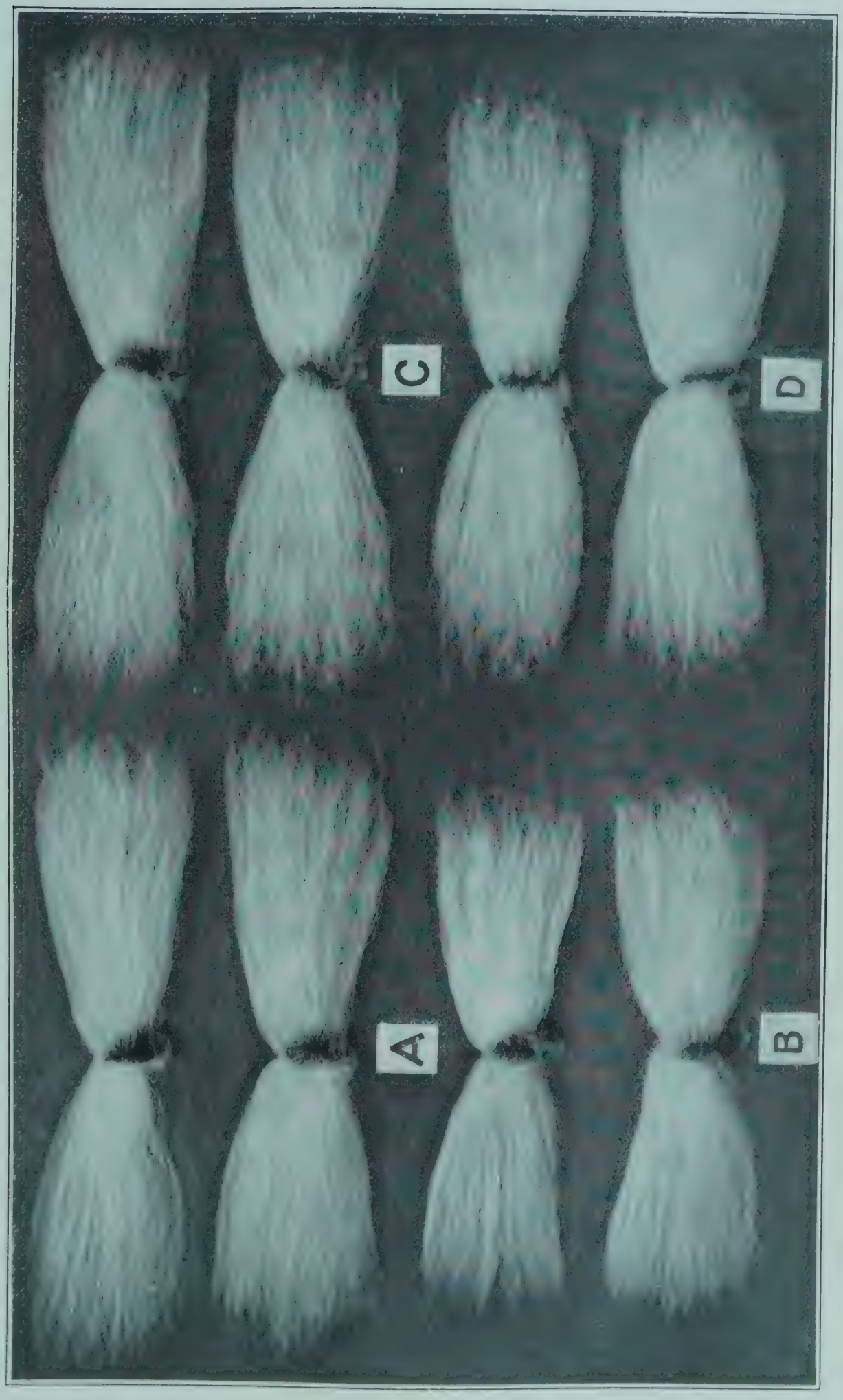
PLATE 49

Continuation of representative bolls, one from each plant of the "parent progeny" as shown in Plate 48.

PLATE 50

Seeds with combed-out fiber of Pima cotton grown in 1919, showing range in length of fiber: A and B from the plants in the progeny of the parent of the variety and C and D from the plants of the commercial stock (strain 5–3) which had the longest and the shortest fiber, respectively. The comparison shows a greater variation in length and a lower minimum length in the immediate progeny of the parent plant than in the present commercial stock.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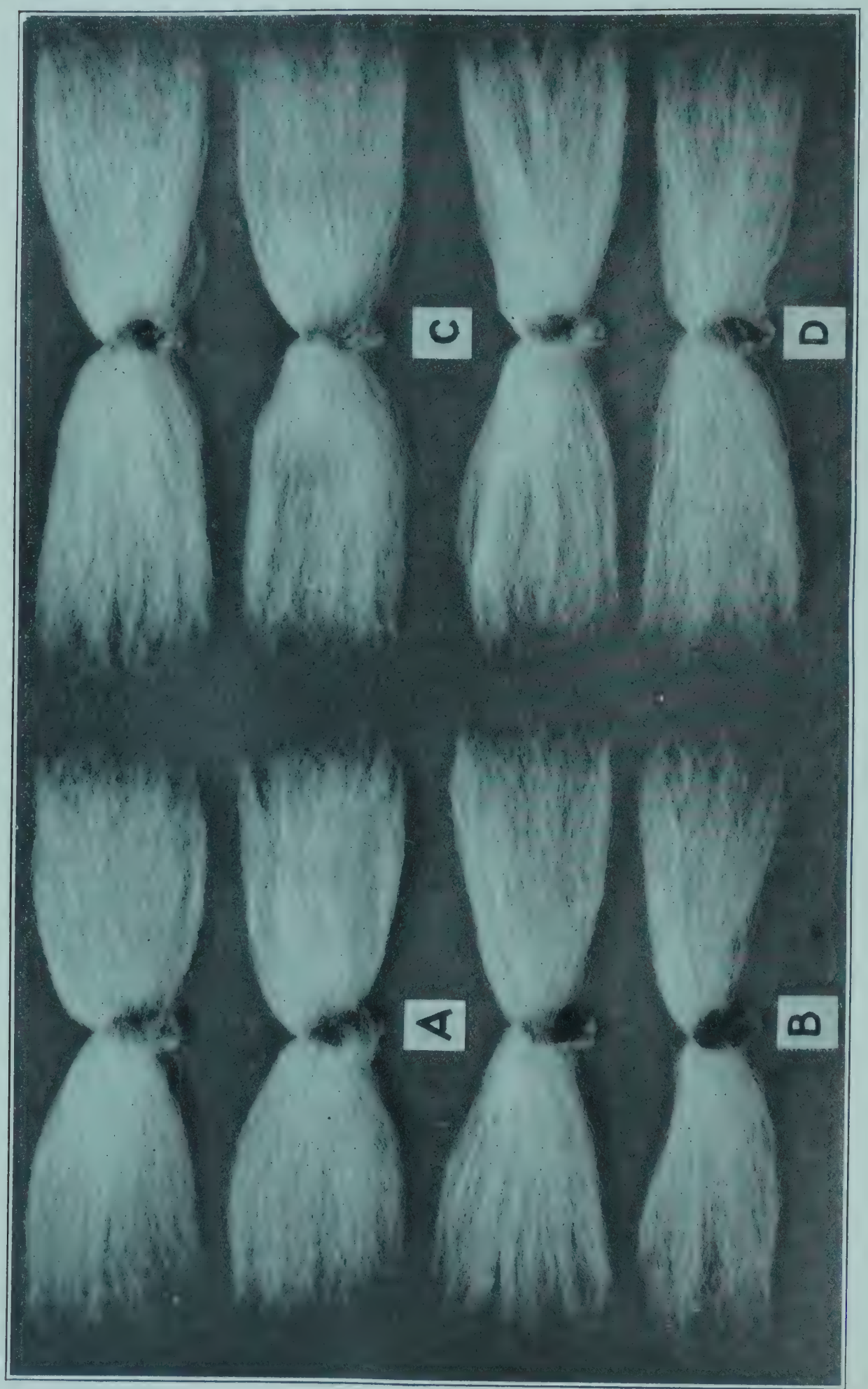
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PLATE 51

Seeds with combed-out fiber of Pima cotton grown in 1919, showing range in abundance of fiber: A and B from the plants in the progeny of the parent of the variety and C and D from the plants of the commercial stock (strain 5–3) which had the highest and the lowest lint index, respectively. The comparison shows a greater variation in abundance and a lower minimum abundance in the parent progeny than in the present commercial stock.

PLATE 52

Seeds of Pima cotton grown in 1919, showing range in fuzziness: A and B from the plants in the progeny of the parent of the variety and C and D from the plants of the commercial stock (strain 5–3) which had the smoothest and the fuzziest seeds, respectively. The comparison shows a greater range in fuzziness and a nearer approach to naked seeds in the parent progeny than in the present commercial stock.

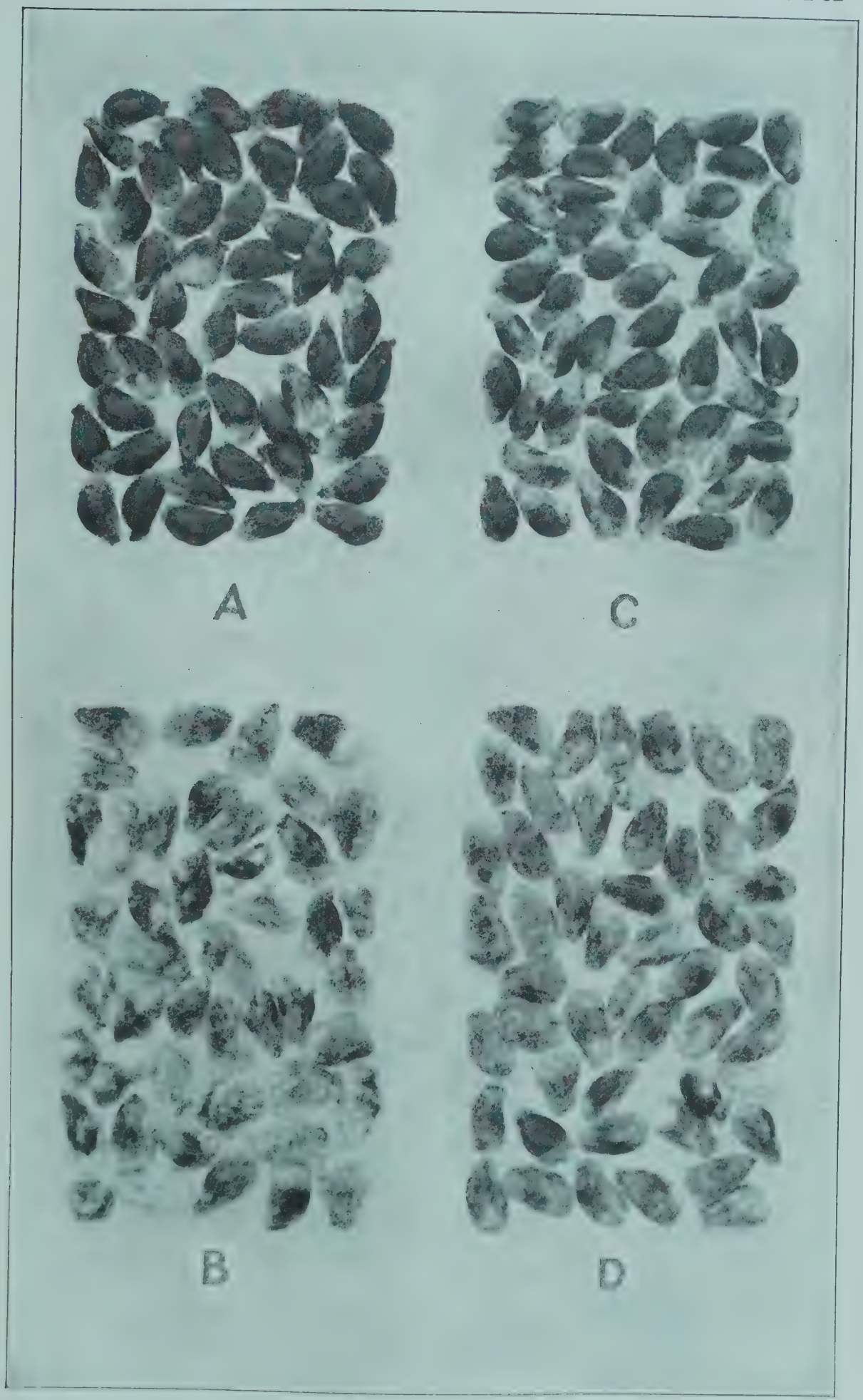
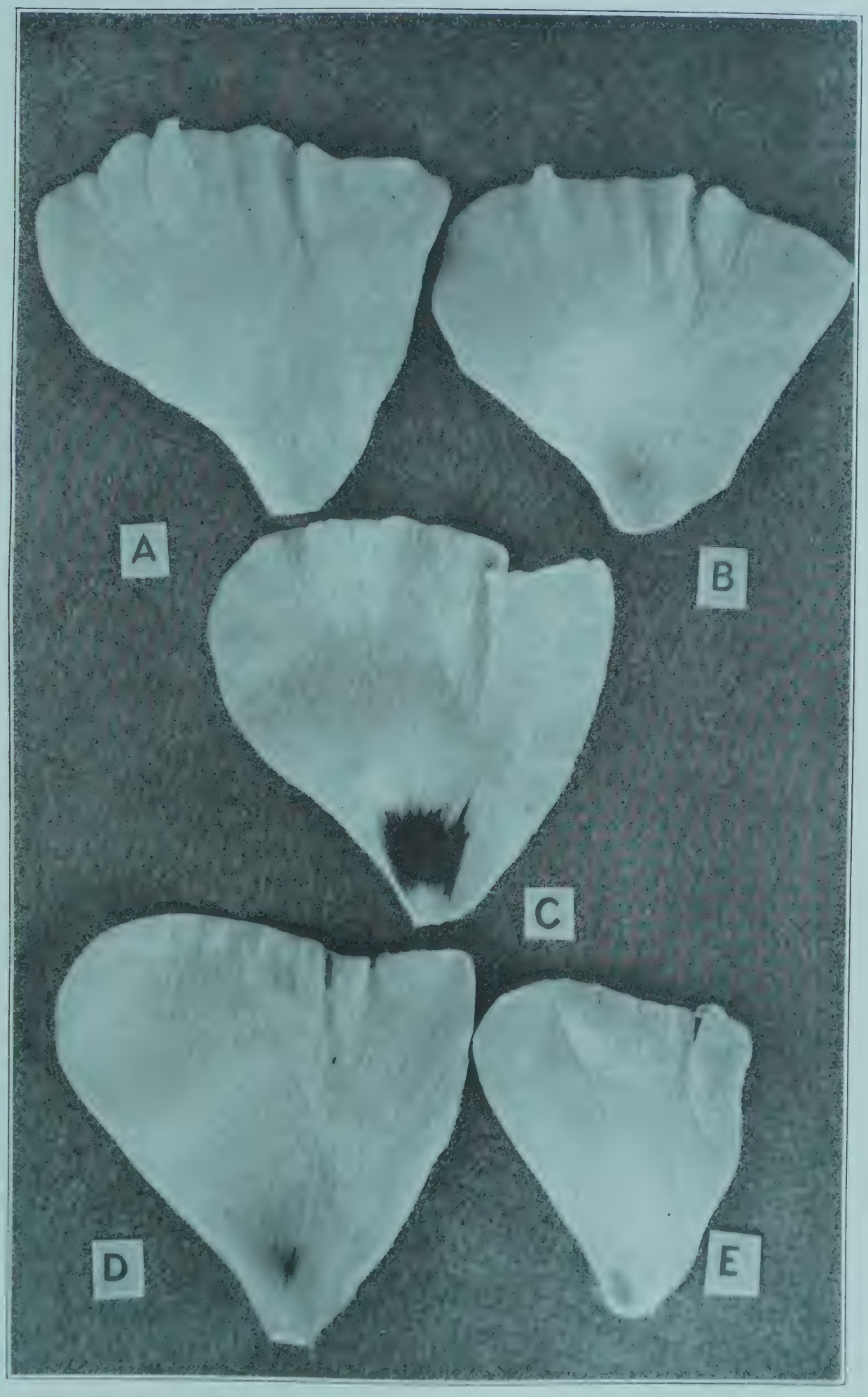

A
C
B
D

PLATE 53

A plant of the Pima variety of American Egyptian cotton, strain 5–3, photographed in August when the lowest bolls were nearing maturity. This plant represents the ideal which is sought in the breeding work with this variety, being free from vegetative branches or "limbs" and having fruiting branches well furnished with bolls at every node from near the base to the top of the stem.

PLATE 54

Comparison of petal-spot development in Pima 4-lock selections (A and B), normal Pima (C), the first generation of a hybrid between Pima Egyptian and the Holdon variety of Upland cotton (D), and Holdon (E). A and B represent the range for the great majority of the Pima 4-lock flowers, from total absence (grade 0) to about grade 2. C represents the full development in Pima (grade 8). D represents a rather weak development for an Egyptian-Upland first-generation hybrid (between grades 3 and 4). E represents the total absence of the spot in Holdon Upland.


A
B
C
D
E

SOME RELATIONS OF TEMPERATURE TO GROWTH AND INFECTION IN THE CITRUS SCAB FUNGUS CLADOSPORIUM CITRI[1]

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INTRODUCTION

In some previous work by the author (*3*)[2] it was found that the results of different inoculation tests on rapidly growing sour-orange trees (*Citrus aurantium* L. (*13*) with *Cladosporium citri* Massee were variable. It had previously been observed in the citrus orchards of Florida that abundant infections from scab did not inevitably follow the presence of abundant moisture on rapidly growing tissue, although these two conditions were usually present when abundant infection did occur. Seasons were encountered when scarcely any infection from scab occurred, even though conditions of moisture and growth appeared to be ideal for an outbreak. The author was led to suspect that temperature was also an important factor in infection.

The experiments which form the basis of this paper were planned to determine what influence different temperatures might have, first, upon infection by the fungus when the two other conditions previously mentioned, abundant moisture on the leaves and rapid growth were maintained, and second, on the growth and spore formation of the causal fungus on culture media. These experiments with the scab fungus carried on during intervals in a more extended temperature investigation with other citrus fungi reported elsewhere (*4*) are seen to be somewhat incomplete, but they appear to throw considerable light on the possible relation of temperature to the occurrence of citrus scab and to offer a more complete explanation for the differences in the occurrence of scab from year to year or from one season to another. It is, of course, realized that in the orchard, under natural conditions, temperature is fluctuating and not constantly maintained as in these experiments. Nevertheless, the experiments indicate at least the comparatively narrow range of temperature within which infection of a very susceptible host is possible under the presumably ideal conditions.

[1] Paper No. 72, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[2] Reference is made by number (italic) to "Literature cited," p. 253.

Citrus scab has been described and illustrated in considerable detail in previous publications by Scribner (*8*, *9*), Swingle and Webber (*14*), Fawcett (*2*), and others. Its main characteristics are corky wart-like projections on leaves, fruit, and small twigs, due to the attack of the fungus on tender, rapidly growing tissue. The portion of the leaves not attacked usually maintains a healthy green color close up to the edge of these warts or scabs.

Considerable confusion has resulted, however, in the identity of the causal organism, *Cladosporium citri* (*1*, *2*). It should be mentioned that the fungus is an unusual Caldosporium and very different from the ordinary type of Cladosporium such as *Cladosporium herbarum* Lk., for example. This causal relation of *Cladosporium citri* to scab was at one time questioned by Grossenbacher (*5*), probably because he was working with another Cladosporium as indicated by the characteristics mentioned in his paper. The author's identification of *Clodosporium citri* as the causal organism has been confirmed by Hesler (*6*), Stevens (*12*), and Horne (*3*), as well as by his own later work (*3*). Moreover, *Cladosporium citri* has been found by Stevens (*10*) to be the cause of a similar disease known as avocado scab in Florida. Sour-orange (*Citrus aurantium*) (*13*) and lemon (*Citrus limonia* Osbeck) trees appear to be the most susceptible hosts to citrus scab. Satsuma orange (*Citrus nobilis* var. *unshiu* Swingle), trifoliata orange (*Poncirus trifoliata* Raf.), and pomelo (*Citrus grandis* Osbeck) are also attacked. Sweet orange (*Citrus sinensis* Osbeck) is nearly immune. In most of the infection experiments here reported, the sour orange was employed because it was one of the most susceptible species.

The experiments were made in 1917 at the Laboratory of Plant Physiology, Johns Hopkins University, in surroundings entirely free from the disease in question. The various temperatures employed were maintained for the most part by means of the apparatus described by Livingston and Fawcett (*7*).[1]

TEMPERATURES AT WHICH YOUNG CITRUS LEAVES WERE INFECTED

A preliminary test on sour-orange seedlings was made on May 16, 1917, with 8-months-old plants, which had been potted about three weeks and were beginning to develop two or three new leaves each. These plants were placed for three days in the different temperatures indicated below and were inoculated by drawing over the surface of the leaves and shoots a camel's-hair brush moistened with spores and bits of mycelium of the fungus in distilled water.

The average temperatures of the nine different chambers were 12°, 16°, 19°, 23°, 26.5°, 31°, 34.5°, 38.5°, and 42.5° C., with a fluctuation of 1° to 2°. One plant was exposed in each chamber for a period of three days in a saturated atmosphere and then taken out and the surface water

[1] The author wishes to acknowledge the aid rendered by Dr. B. E. Livingston in these experiments.

allowed to dry. The plants that had been inoculated at 16°, 19°, and 23° subsequently became diseased. The plant from the 16° chamber had scabs just visible 7 days from date of inoculation. Those from the 19° and 23° chambers were not seen to be diseased until 10 days later. The plants that had been inoculated at 16° and 19° had many scabs. The plant that had been inoculated at 23° was only slightly diseased even on the seventeenth day after inoculation.

Controls which were not inoculated, but on which distilled water was drawn over the leaves, were subjected to 16° and 26.5° C. and kept under the same conditions as the others, but no scab developed upon them.

A second test on sour-orange seedlings just like the first was made on June 5, 1917, except that different temperatures were maintained in some of the chambers and two plants were left in each temperature, and they remained for 54 hours instead of 3 days. Control plants not inoculated were used at 20° and 27.5° C. The average temperatures in this test were 14°, 20°, 24.5°, 27.5°, 32.5°, 36°, 40°, and 44.5°.

In this test one of the plants inoculated at 20° C. developed scabs on its leaves. These were first noted two weeks after inoculation. None of the other plants developed scabs. As will be seen in Table I, the chamber at 20° is the only one that lies in the range at which infection occurred in the other two tests, namely, 16° to 23°. The chambers at 14° and 24.5°, therefore, appeared to be outside, one below and the other above, the range for infection.

On September 24, 1917, a third inoculation test was made with sour-orange trees in pots, about one year from seed, on which small new leaves were developing. The plants were surrounded by cylinders of blotting paper kept moist by absorption of water from below. Another piece of blotting paper was placed over the top of the cylinder. The different temperature chambers (7) in which the plants were left for 3 days were maintained nearly constant within a fluctuation of about 0.5° C. At the end of this 3-day period when they were taken out, the moist cylinders of blotting paper were removed, and the plants were set in the greenhouse. In about ½ hour the moisture had evaporated from the surfaces of the plants. At the same time that these were taken out, two other plants were inoculated with the fungus as before and were set in the same greenhouse and under the same conditions as the others. These were not surrounded by blotting paper but were allowed to dry like the others, after water with spores had been drawn over their leaves.

The temperatures at which the plants remained during the 3-day period in the saturated atmosphere of the dark chambers were 13.5°, 16°, 18.5°, 21°, 24.5°, 27.5°, and 32° C., respectively.

The temperatures recorded in the greenhouse for the 12 days after the plants were taken out of the temperature chambers gave an average minimum of 15° C., and an average maximum of 30°.

Only those subjected to 16°, 18.5°, and 21° C. for the 3 days after inoculation developed any scab on their leaves after removal to the greenhouse benches.

On plants inoculated at 16° C. scab was just visible in 6 days from the time of inoculation, or 3 days from the time of removal from the temperature chamber. In 2 weeks the scab lesions were well marked.

Plants inoculated at 18.5° C. showed a slight indication of scab 5 days after inoculation, or 2 days after plants were removed from the temperature chamber. In this case the scab lesions were well marked in 11 days from time of inoculation.

Plants inoculated at 21° C. showed a slight indication of scab 5 days after inoculation, as was true of those at 18.5°. In 7 days the diseased spots were quite distinct, and in 11 days they had developed into definite and typical scabs. On plants inoculated at 13.5°, 24.5°, 27.5°, and 32°, however, no sign of lesions developed.

Control plants were also kept under the same conditions, but none of these developed scab lesions at any time. The two plants which were inoculated with the fungus at the time the others were taken out of the temperature chambers and from whose surfaces the water was allowed at once to evaporate also remained free from scab. These were considered to be additional controls. The nonappearance of scab on these two plants indicated that infection at exposures of 16°, 18.5°, and 21° C. developed because the hyphae had penetrated while the plants were still in the temperature chambers. This conclusion is also supported by the fact that the time from inoculation to the appearance of visible scabs agrees well with that observed in previously reported tests (*2*, *3*). The time elapsing between the removal of the plants to the greenhouse bench and the first appearance of scab appears generally to be too short to represent the incubation period of the fungus within the tissues of the host.

On September 28, 1917, a set of young pomelo seedlings with leaves just begining to unfold was inoculated in the same manner as that described for the sour-orange seedlings of the third test, except that these were left four days in the temperature chambers instead of three. With the exception of one leaf on a plant inoculated at 18.5° C., which developed a slight indication of disease but no distinct scab, there was no visible sign of disease on any of the plants up to October 17, when the last observation was recorded.

In all four tests just considered, scabs were always confined to rapidly growing leaves. The older leaves always remained free from the disease in every test. Neither was there any development of scabs on any of the large number of similar plants kept in the greenhouse and not inoculated.

TABLE I.—*Range of infection as indicated by the four inoculation tests on citrus seedlings*[1]

Plant and date of inoculation.	Results of inoculation at temperature (°C.) of—																			
	12	13.5	14	16	18.5	19	20	21	23	24.5	26.5	27.5	31	32	32.5	34.5	36	38.5	40	42.5	44.5
Sour orange:																					
(1) May 16, 1917	o	....	...	*	....	*	...	...	*	....	o	....	o	...	....	o	...	o	...	o	....
(2) June 5, 1917	...	....	o	...	....	...	*	...	...	o	....	o	...	...	o	....	o	....	o	....	o
(3) Sept. 24, 1917	...	o	...	*	*	...	...	*	...	o	....	o	...	o	....	....	...	....	...	....	....
Pomelo:																					
(4) Sept. 28, 1917	...	o	...	o	*	...	...	o	...	o	....	o	...	o	....	....	...	....	...	....	....

[1] * Indicates definite infection; o, no infection.

INOCULATION OF DETACHED LEAVES

Young sour-orange leaves were detached and placed in Petri dishes, one set containing distilled water on which the leaves were floated and the other set containing cornmeal agar. Some of the fungus mycelium was placed in contact with the leaves, and the preparations were allowed to remain in the temperature chambers for 15 days. The temperatures used were 13.5°, 16°, 18.5°, 21°, 24.5°, 27.5°, and 32° C., with a fluctuation of about 0.5°. Infection took place in water at 16°, 18.5°, 21°, 24.5°, and 27.5°, but not at 13.5° nor at 32°; and in cornmeal agar at 18.5° and 21°, but not at 13.5°, 16°, 24.5°, 27.5°, nor 32°.

INFLUENCE OF TEMPERATURE ON GROWTH AND SPORE PRODUCTION OF CULTURES

In addition to the inoculation experiments described above, the rate of growth and the formation of spores were studied with different maintained temperatures. Distilled water, in which was floating a young sour-orange leaf, and cornmeal agar were used as culture media. Inoculation was accomplished by means of a disk (2.5 mm. in diameter) of agar medium bearing the mycelial weft. The cultures were kept in the dark, maintained temperature chambers for three or four days. At the end of the period the radial extension of the hyphae from the transferred disk, was determined. Observations were also made on the general abundance of spores. The data obtained are brought together in Table II. The growth rate is seen to have been very slow as compared with that of many other citus fungi (*4*). For the time employed and for the temperatures used the greatest extension of hyphae occurred at 21° C. This growth rate was smaller, for each temperature, in cornmeal agar than in distilled water with the leaf, except at the two higher temperatures. At 32° no extension of hyphae was seen in water, while in cornmeal agar the enlargement was about one-sixth of that obtained at 21° for the same period.

Spores were observed on the marginal hyphae in from 24 to 48 hours in all cases where growth was observed, except in the agar culture at 32° C. The first examination for presence of spores was made after 48 hours in the agar cultures. Spores were abundant at the first examination upon the growing hyphae of all the test cultures at 21°, one of the temperatures

at which infection of rapidly growing sour-orange seedlings and detached sour-orange leaves had been observed to be pronounced. Spores were also abundant in the water preparations at 24.5° and 27.5° after 24 hours, and in the preparations of agar at 24.5° after 48 hours; at these temperatures no infection had occurred in the tests with growing plants, but a slight infection had been observed after 10 days on detached leaves in water.

Spores that had fallen from the hyphae in certain ones of these cultures were found to have fallen on the surface of the medium at some distance laterally from the ends of the aerial hyphae on which they had been borne. Apparently these spores had been ejected with considerable force from the ends of the hyphae. In one case at 16° C. a fringe of spores was observed, most of them at least 210 microns distant from the ends of the outermost hyphae. This feature of spore dispersal in cultures had previously been seen but not recorded. It was noted as most frequent after four days in cultures growing at the edges of leaves in cornmeal agar at 16°, 18°, 21.5°, and 24.5°.

In the 16° preparation with water and a sour-orange leaf, spores were observed to be mostly formed on the tip ends of the outwardly extending aerial hyphae at some distance from the floating leaf. They were nearly hyaline at first, subsequently becoming slightly dusky. Most of them were 1-celled, but a few were 2-celled. Detached spores were germinating from the ends of spores in line with the longest axis. Hyphae were hyaline when viewed singly, but were pinkish or flesh-colored in mass. On cornmeal agar under the same conditions spores were forming with from two to four in a chain, and the ejected spores were germinating on the surface of the medium.

The influence of temperature on the vegetative hyphae was marked. At 27.5° and 32° C. in the cornmeal agar preparations the hyphae were broad and straight, 8 to 12 microns or more in diameter. At 23° and 21°, on the other hand, the hyphal diameter was only one-half as great and the hyphae were more bent and tortuous. At 18.5°, 16°, and 13.5°, the hyphae were broader, much as at the higher temperatures.

TABLE II.—*Growth and spore formation in Petri-dish cultures of Cladosporium citri at different maintained temperatures*

Temperature.	Fungus floating in water with tender leaf.			In cornmeal agar.		
	Radial growth in 3 days.	Spore formation.		Radial growth in 4 days.	Spore formation.	
		In 1 day.	In 3 days.		In 2 days.	In 4 days.
°C.	*Microns.*			*Microns.*		
13.5	62	Few.....	Few.....	38	Few.....	Few.
16	170	...do.....	Abundant..	92	...do.....	Do.
18.5	285	...do.....	Few.....	116	...do.....	Do.
21	655	Abundant..	Abundant[1].	370	Abundant[1].	Abundant.
24.5	385	...do.....	Abundant..	323	...do.....	Do.
27.5	154	...do.....	Abundant[1].	197	Few.....	Few.
32	0	None.....	None.....	62	None.....	None.

[1] Spores found some distance laterally from ends of the outermost hyphae as if ejected with considerable force.

TABLE III.—*Number of days from inoculation to first indication of disease at different temperatures* [1]

	Days after inoculation at temperature (°C.) of—								
	16	18. 5	19	20	21	23	24. 5	26. 5	27. 5
Sour-orange seedlings:									
(1) May 16, 1917	7	—	17	—	—	17	—	—	—
(2) June 5, 1917	—	—	—	14	—	—	—	—	—
(3) Sept. 24, 1917	6	5	—	—	5	—	—	—	—
Detached leaves:									
(4) In cornmeal agar	—	5	—	—	4	—	—	—	—
(5) In water	6	4	—	—	3	—	10	—	10

[1] A dash indicates that no scabs developed on plant placed at the respective temperatures. A blank space indicates that no plants were used in a given test at these respective temperatures.

DISCUSSION

Infection of sour-orange seedlings was limited to a range of about 8° C., 16° to 23°, inclusive, in these experiments. This range could not have been over 10° in extent under the same conditions even if more temperatures had been investigated, since plants inoculated at 14° and at 24.5° failed to become diseased. This range is seen to include the temperature (21°) at which the fungus was found to have the most rapid growth in water and in cornmeal agar medium. The temperatures just outside the range on the lower side (14° and below) are those at which the fungus grew somewhat more slowly than it did at the temperatures just outside the range on the upper side (24.5° and above). If, however, we consider the experiment with detached leaves in water (Table III) in which an exposure of 15 days was given at the different temperatures, the range for infection, though not extended downward, was extended upward to 27.5°, a temperature at which the hyphal growth in water was approximately the same as that at the lower limit of the range, 16° (Table II). This may indicate that the rate of extension of the hyphae must be above a certain minimum before infection of the host can occur, a point that may have some significance. The rate of extension of the hyphae also appears to have a relation to the time between inoculation and first appearance of scab as is shown, in a general way, in Table III. This time period, with a single exception, is shortest with temperature at or near 21°, the temperature for maximum growth if the tests on different dates be considered separately.

It is, of course, not to be definitely concluded that infection can not occur with temperatures outside the range within which it was confined in these experiments, but it appears probable that scab is not to be expected with temperatures that remain outside this range. Of course, orchard temperatures may vary so as to subject the trees to temperatures within the range of infection for a sufficient time period to allow the penetration of the fungus. No attempt was made to determine

the minimum time necessary for the fungus to become established in the host tissue at the different temperatures listed in order to produce scab subsequently.

The interrelation of the host and parasite also demands consideration. Temperature, of course, influences the host as well as the parasite, and the lack of infection in these experiments at certain temperatures may have been due to special physiological conditions or states either in the host or in the parasite, or even in both. It may be suggested that there was not enough time for infection in those cases where it failed to occur. From the experiments in which leaves floating in water were left for a long time in contact with spores of the fungus at different maintained temperatures, it appears that, even with long periods of time, there are certain temperatures, not fatal to either parasite or host, at which infection does not take place. At some temperatures the fungus even grew fairly well on the surface of the leaf without producing infections leading to scab.

The relation of temperature to infection by the scab fungus, as here brought out, seems to suggest a satisfactory explanation for some differences in the results of inoculations previously recorded by the author (*2*). A set of inoculations made in August, 1906, failed to produce scab, while a similar set made in January, 1909, was successful. An examination of the weather records for the locality where the experiments were made shows that the mean daily temperatures for the two weeks following the August experiment fluctuated between a minimum of 23° and a maximum of 30° C., while the corresponding temperature range for the January experiment lay between a minimum of 11° and a maximum of 19°. In the former case, the daily means were above the range for infection of seedlings in the experiments here reported except at its minimum, 23°, while the other case shows an overlapping of the range of daily means with the range for infection, to 19°. Since the temperatures in the greenhouse where the 1909 experiments were performed were probably higher than those outside, the successful infections in January were almost certainly due to the fact that the temperatures fell for the most part entirely within the range that is favorable to infection.

The temperature relation here emphasized is probably important in determining the occurrence of scab in the orchard and in the explanation of scab epidemics. But temperature is only one of a number of conditions, such as stage of growth, humidity, time period, etc., that must be fulfilled for scab to occur.

Among the various conditions discussed by Grossenbacher (*5*) as favoring the development of scab, the following general observations seem to bear on this temperature relation.

If the air is fairly dry and the weather mostly warm and bright during the development of the first spring flush, scab may fail to develop. On the other hand, the disease

often becomes very severe in groves if the weather is cold and wet during the development of the first spring growth.

Again:

Some trees in scabby groves under observation during the past two years have retained the late starting habit and remained practically free from the disease.

Again:

During the early spring of 1915 the air was very moist and cold while the first growth was in its early stages and as a result sour scab developed in great abundance even on high sandy land if growth was early and vigorous.

Stevens (*10*) says of citrus scab:

If cool wet weather prevails at the time the new growth is putting out or at the time the fruit has set, the disease is apt to be severe . . . in groves where scab has become established.

In the light of these temperature experiments, the greater severity of scab at the low spring temperatures of Florida seems to be due largely to the fact that these temperatures fall mainly within the range for infection, while the later spring and summer temperatures are usually too high for infection to take place, even though other conditions are favorable. An examination of the temperature data for Tampa, Fla., given in Table IV suggests that this last statement may be translated into terms of mean temperatures, to the effect that severe infection will usually occur when the mean temperatures are well within the infection range and that infection will be unlikely to take place when the mean temperatures are outside this range.

TABLE IV.—*Mean temperatures at Tampa, Fla.*

[In degrees centigrade]

Month.	1909	1910	1911	1912	1913	1914	1915	1916	1917	1918
February	16.5	15.5	17.5	13.5	18.5	15.5	15.5	16.5	16.5	20
March	19.5	19	20	20.5	21	16.5	14.5	17	20.5	22
April	23	20.5	23	23	21	22	20.5	20.5	22	22
Mean	19.5	18.5	20	19	20	18	17	18	20	21
May	24.5	24.5	24.5	25.5	24	25	26	25	24	24.5
June	27	26.5	27	26	26	27	28	26	26.5	27
July	26.5	26.5	27	28	27	27	28.5	28	28	28
Mean	26	26	26	26.5	25.5	26.5	27.5	26.5	26	26.5

The average of the monthly mean temperatures for February, March, and April for this 10-year period was between 17° and 21° C., while the average of the monthly mean temperatures for May, June, and July was between 25.5° and 26.5°. All the monthly mean temperatures for the first period were well within the infection range for scab, while all the

monthly means for the second period are well above the infection range as determined by the inoculation experiments.

It is of interest to note also that the lowest average for February, March, and April was 17° C. in 1915, the year mentioned by Grossenbacher (5) as one in which scab developed in great abundance on the growth starting in early spring. These data taken in connection with the maintained temperature experiments seem to explain the frequent absence of scab on tender growth of the second or third cycles even in the moist periods of summer. The spores may be present, the moisture and growth conditions may be favorable, but the temperature may be too high.

The occurrence of severe late scab in some seasons is probably due to a decided drop in the temperature, bringing it well within the infection range for a sufficient length of time for infection.

SUMMARY

(1) Experiments to determine the influence of temperature on scab infection on young sour-orange (*Citrus aurantium*) leaves and on the growth and spore formation of the causal organism (*Cladosporium citri*) are reported.

(2) The inoculation temperatures resulting in infections of growing plants under conditions of rapid growth and abundant moisture were 16°, 18.5°, 19°, 20°, 21°, and 23° C. No infections were obtained under the same conditions on plants inoculated at 12°, 13.5°, 14°, 24.5°, 26.5°, 27.5°, 31°, 32°, 32.5°, 34.5°, 36°, 38.5°, 40°, 42.5°, and 44.5°.

(3) Detached leaves floated in water with the scab fungus were infected at 16°, 18.5°, 21°, 24.5°, and 27.5° C.

(4) The temperature at which the greatest extension of hyphae of the causal organism in cultures was observed was 21° C. The highest temperature at which extension was observed in water was 27.5°, and in cornmeal agar 32°.

(5) Spores were observed in 48 hours or sooner in all the temperatures at which growth took place except 32° C. At certain temperatures the spores appeared to be ejected with considerable force from the ends of the hyphae.

(6) The temperature at which the time was shortest between inoculation and first observance of signs of disease was usually 21° C. in the different tests. This time increased toward the upper and lower limits of the infection range.

(7) This limited range of temperature at which infection of a susceptible host took place under the presumably favorable conditions of the experiment appears to explain the great differences observed in the occurrence and severity of scab from year to year and from season to season in citrus orchards. It also explains the differences in results of previous inoculation experiments not hitherto understood.

(8) The conditions necessary for scab infection indicated by these experiments are (1) viable spores of the fungus, (2) young citrus leaves of a susceptible species, (3) moisture, and (4) temperatures between 16° and 23° C.

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BACTERIAL WILT OF CASTOR BEAN (RICINUS COMMUNIS L.)

By ERWIN F. SMITH, *Pathologist in Charge, Laboratory of Plant Pathology*, and G. H. GODFREY, *Pathologist, Office of Cotion, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*[1]

HISTORY AND DISTRIBUTION

The bacterial wilt of castor beans was first brought to the attention of the writers in May, 1918, when specimens of diseased plants were received from Townsend, Ga., where they were collected by Mr. I. C. Jagger. Isolations made from this material were used immediately for cultural studies and for numerous inoculations as already reported.[2] Soon after the first observation of the disease in the field, by direction of the Office of Cotton, Truck, and Forage Crop Disease Investigations, a survey was undertaken by Mr. Jagger of castor bean plantings in the South, particularly in Florida. The survey extended over a period of about two weeks and covered practically all sections of the State of Florida. In addition, reports of the occurrence of the disease were received from other men in Florida and other States.

The disease occurred at or near the following points in Florida: Monticello, Jacksonville, St. Leo, Ocala, Orlando, Plant City, Tampa, Lucerne Park, Winter Haven, Mulberry, Fort Meade, and Frostproof; at Townsend, Ga.; and at Dothan, Ala. The loss varied considerably in these localities, very exceptionally exceeding 10 per cent and varying from that down to zero. The highest reported loss was 30 per cent in a field in southern Georgia.[3]

On a trip made by the junior author early in August, freshly wilted plants were still to be found in the fields. During this survey the wilt was observed definitely at Orlando, Tampa, Seffner, and Fort Meade, Fla., and at Dothan, Ala. It is very likely that the disease occurred also in North and South Carolina and in Mississippi. The descriptions given by farmers of an early disease on the plants indicated this, but no authentic material was received.

A thorough search by both Mr. Jagger and the junior writer at different times among the heavy plantings along the east coast of Florida, from Miami to New Smyrna, failed to show the disease to be present. These observations were made at Miami, Little River, Arch Creek, Davie, West Palm Beach, Vero, Deer Park, Melbourne, Titusville, Mims, and New Smyrna.

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[1] The field observations on this disease were wholly in the hands of Mr. Godfrey. The identification of the organism was made by the senior writer, and the inoculations were under his direction.

[2] SMITH, Erwin F., and GODFREY, G. H. BROWN ROT OF SOLANACEAE ON RICINUS. *In* Science, n. s. v. 48, no. 1228, p. 42. 1918.

[3] Reported by Mr. T. B. Young, of the Office of Drug Plant Investigations.

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The east coast section differs from the interior and west coast sections of Florida rather strikingly in that an abundance of lime is present in the soil, cropping out here and there. Therefore this type of soil is probably in the main alkaline, as opposed to the more or less acid soil of the pine flats and the more rolling pine lands of the interior. It is suggested, therefore, that further studies of the distribution of *Bacterium solanacearum* EFS be made in Florida with special types of soil in mind to see if there is anything in this suggestion.

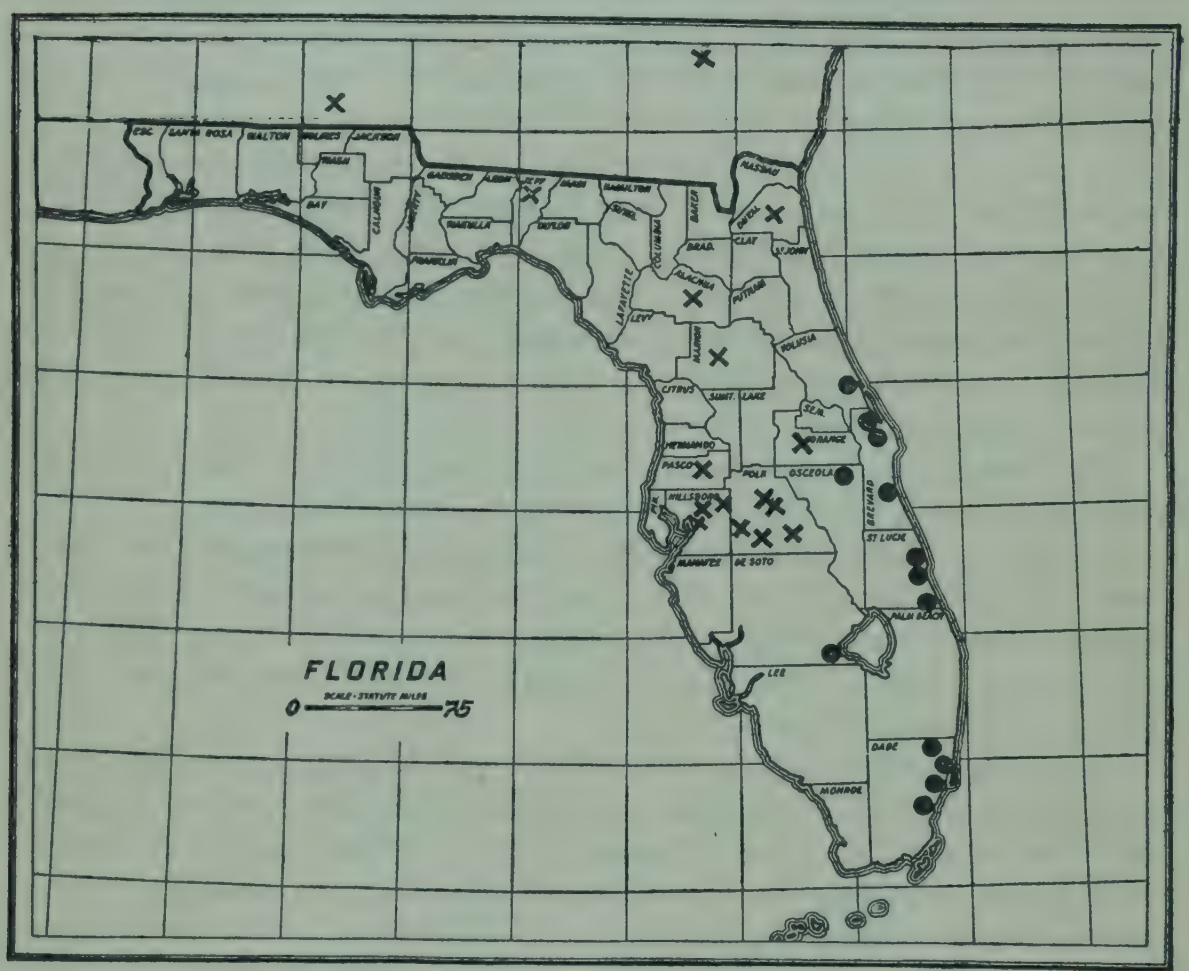

FIG. 1.—Map showing distribution in Florida in 1918 of Ricinus wilt due to *Bacterium solanacearum*. X indicates localities where the disease was found; ●, localities where the disease could not be found.

Late in the season plantings in the Everglades muck land, in the vicinity of Moorehaven, were inspected without any signs of the wilt being found. The outline map (fig. 1) shows the distribution of the wilt in Florida, as well as the points where the disease was searched for and not found, showing the sharp division of the State into two distinct regions.

Another observation on the soil type and its apparent relationship to wilt, as reported by Mr. Jagger, is to the effect that the newer land was more likely to show the wilt than land that had been longer under cultivation. This was observed on numerous occasions in the sections where wilt occurred. One or two particular cases may be mentioned.

On one field of 295 acres at Ocala, Fla., this contrast was particularly striking. No wilt whatever was observed in the main part of the field, which had been under cultivation for many years. On a new section of the field, which had been cultivated only two years and which adjoined pine woods, wilt occurred in varying amounts, running from one-tenth of 1 per cent to 10 per cent in different spots. It was typical rolling pineland with little humus. The older land was originally the same but had developed considerably more humus and a darker color.

A field at Frostproof, Fla., was inspected on June 6. This field was made up entirely of new land cleared and plowed in January and February, 1918, and planted in March. The wilt occurred in certain areas, especially in the lower portions of the field, to the extent of 5 to 10 per cent. Several other fields in the same general locality, which had been under cultivation for several years, showed a complete absence of the wilt.

At Mulberry, Fla., a plot of 80 acres of low pineland, with saw palmetto in the lower spots, was cleared in the latter part of February and planted March 13. While this gave an excellent stand of castor beans, wilt occurred throughout to the extent of one-tenth of 1 per cent to 5 per cent. A few square rods planted early in May (very late) showed numerous wilted plants.

The junior writer likewise inspected a field in Dothan, Ala., in which the only wilt to be found was in a newly cultivated end of the field adjoining pine woods.

The senior author has frequently observed this disease in tomatoes grown on "new land" in Florida.

Due to the fact that there were no extensive commercial plantings in 1919, the only observations on the disease in 1919 were in some experimental plantings made by the junior author at Orlando, Fla. As early as April the first cases of wilt were to be found, and from that time on till at least June 16 new cases were appearing almost daily. These cases all occurred on sandy land which had not been under cultivation for some years.

SIGNS OF THE DISEASE IN THE FIELD

The signs of the bacterial wilt on Ricinus are, in general, the same as on other plants described in detail by Dr. Erwin F. Smith, in "Bacteria in Relation to Plant Diseases,"[1] and in earlier publications. The disease in this plant, as in the Solanaceous plants, is a true wilt of the green leaves and growing points, without previous yellowing or other partial or complete discoloration (Pl. 55). It may occur when the plants are small, only 2 or 3 inches high, in which case it is likely to prove fatal. It is also found in plants of all sizes up to several feet high. In smaller plants a single leaf may wilt, and the vascular system near the base of the plant

[1] SMITH, Erwin F. BACTERIA IN RELATION TO PLANT DISEASES. v. 3. Washington, D. C. 1914.

on that side may show internal browning, or again the entire plant may droop. The wilting may come about very suddenly or slowly. The first manifestation of wilt often appears after a period of wet weather of several days' duration, such weather apparently favoring infection. More of the wilt is seen in hot, dry weather. Often a plant may recover temporarily during moist weather or during a cool night after a hot, dry day, which has resulted in the appearance of wilt. Sometimes this recovery leaves a shriveled tip or margin on some of the leaves. The ultimate result of the wilt, in the more severe cases at least, is the complete death of the plant. After wilting the leaves dry and turn black, later dropping from the plant and leaving the bare black stalk and branches.

In many plants infection was present late in the season but was not severe enough to cause permanent wilting or death of the plant. In such cases marked dwarfing occurred. In a field at Dothan, Ala., it was easily possible to pick out infected plants that showed no external signs other than dwarfing. Plate 55, A, reproduced from a photograph taken at Dothan, Ala., on August 24, 1918, shows from left to right, a dwarfed and a wilted plant (both of which showed unmistakable internal signs of the bacterial wilt) and a large normal plant.

A disease due to a root trouble and entirely distinct from the wilt, but showing some signs similar to it, was found in the vicinity of Miami, Fla., and at other points especially in muck lands. These plants appeared to be affected by asphyxiation of the roots due to too high a water table. Many such plants were observed with the root systems entirely dead and often with the tap roots rotted off 3 or 4 inches below the surface of the ground. Such root conditions resulted in the death of the top of the plant, manifested by wilting and drooping of the leaves and twigs and finally by the death and blackening of the entire plant (Pl. 56).

The bacterial wilt can be distinguished easily from the disease described above and from any other known disease of the castor bean by the typical browning of the vascular system near the base of the plant. In the severer cases this browning is seen in the entire vascular ring, penetrating more or less deeply into the woody part of the stem. In cases of light infection, such as in the dwarfed plants mentioned heretofore, the browning may be seen in only a few bundles, all possibly on one side of the stem. In fact, cases were observed in which only a single bundle gave this manifestation of the presence of disease. In addition to the browning it is always possible with the aid of a good hand lens to observe drops of bacterial exudate at the freshly cut ends of the vascular tubes, particularly if the plant is still green. Sometimes it is necessary to pinch the end of the stem to force these drops to the surface.

ISOLATION OF THE CAUSAL ORGANISM

Such materials as have just been described will almost invariably produce pure cultures of the causal organism when proper precautionary measures against external contamination are taken. Repeated cultures were made by flaming momentarily the green stalk from a wilted plant, then cutting it off with a sterile scalpel and squeezing out by means of strong forceps a drop of the plant juice together with the bacteria into the tube of beef agar. From this, dilutions were made and then plates were poured. This was varied by sterilizing the surface of a stalk with mercuric chlorid 1 to 1,000, girdling it with a circular cut and breaking it off, after which sterile conditions were still further insured by searing the edge of the freshly made cut with a red-hot scalpel. A hollow could now be made safely in the end of the stalk and a few loops of beef broth inserted and mixed with the plant juices. From this very thick suspension of bacteria, dilutions were then made for the poured plates.

RESULT OF INOCULATIONS

As soon as results from the poured plates gave indications that the organism in the diseased Ricinus plants was *Bacterium solanacearum*, inoculations were made into tomato plants, which wilted promptly. Cultures in other media such as nitrate bouillon (where reduction took place) and pink litmus milk (which became and remained blue) also served to confirm the diagnosis. From the interior of one of the wilting tomato plants Petri dish agar poured plates were made and subcultures from some of the colonies were tested out on other tomatoes which also wilted (Pl. 57).

By this time Ricinus plants large enough to inoculate were available, and a series of inoculations were made by needle on the hypocotyls. On these plants the progress of the disease was slower than on the tomato, but there was profound dwarfing as a result of the inoculations, and subsequently the plants wilted with bacterial occupation and browning of parts of the vascular system (Pl. 58–60). From such plants also the organism was plated out in practically pure culture. Large Ricinus plants also were successfully inoculated by needle pricks (Pl. 61). Subsequently the disease was produced on Ricinus by breaking some of the roots in soil infected by burying in it Ricinus plants already inoculated and swarming with the bacteria (Pl. 62).

We also obtained successful inoculations on a series of jimson weeds (*Datura stramonium* L.), the phenomena in which corresponded exactly to results formerly obtained by the senior writer with *Bacterium solanacearum* plated from other plants—that is, wilting, vascular infection, brown stain, etc.

The Ricinus organism was also inoculated successfully into the common nasturtium (*Tropaeolum majus* L.), another plant subject to *Bacterium solanacearum*.[1]

Subsequently it occurred to the senior writer to try out the organism on cotton, vanilla, and sunflower—three plants hitherto untried.

Cotton plants when of any size proved resistant, but the young seedlings are subject to the disease. Thus, if inoculations are made by needle pricks into the hypocotyl soon after the plants appear above ground they are first profoundly dwarfed (Pl. 63) and then wilted and shriveled (Pl. 64). Bacteria were then found in enormous numbers, at least in the parts above ground, the needle pricks being made near the cotyledons. These results were obtained in 1918, and the same results were obtained again in 1920, using *Bacterium solanacearum* plated from wilting North Carolina tobacco (the Granville tobacco wilt).

The common vanilla (*Vanilla planifolia* Andrews) also contracted the disease. Under our hothouse conditions it was possible to produce wilt and brownrot only of the softer growing tips of the shoots (Pl. 65), but the bacteria were traced a considerable distance farther in the vascular system, and it seems likely that under tropical conditions whole plants might be subject to the disease. A few plants only having been inoculated in 1918 (always by delicate needle pricks), the inoculations were repeated in 1920 with the same results, using *Bacterium solanacearum* obtained from North Carolina tobacco. These results were wilting and rot of the terminal shoots in about a week or 10 days' time, brown to black stain in the tissues, with multiplication of the bacteria in the vessels (and in the parenchyma of the softer parts); and from the interior of one of these stems a pure culture of the right organism was reisolated.

Sunflowers (*Helianthus annuus* L.) inoculated in the soft stems when about one-fourth grown proved very susceptible. The culture used for this purpose was a subculture from a poured-plate colony isolated from diseased vanilla which was infected from a poured-plate single colony subculture from a diseased tomato which had been inoculated in the same way from a diseased Ricinus. The plants wilted in a week or two with the usual brown staining of the vascular system, and enormous numbers of the bacteria developed in the vascular system to long distances from the point of inoculation, which was near the top of the plant, so that when cross sections of such stems were made the bacteria oozed out copiously (Pl. 59, B), as a gray white slime even down to the surface of the ground. Here also there was a profound dwarfing. The Ricinus organism was reisolated from an inoculated diseased sunflower on Petri dish agar poured plates, and with a subculture from one of the colonies tomatoes were again successfully inoculated (Pl. 66).

[1] BRYAN, Mary K. A NASTURTIUM WILT CAUSED BY BACTERIUM SOLANACEARUM. *In* Jour. Agr. Research, v. 4, no. 5, p. 451. 1915.

Stanford and Wolf [1] having reported successful inoculations of *Bacterium solanacearum* on the common garden balsam (*Impatiens balsamina* L.), we tried the Ricinus organism on these also with marked success, the tops wilting and the brown streaks, due to stained vascular bundles, showing through the translucent stems for long distances down, the inoculation being made near the top (Pl. 67, A). In such cases a microscopic examination showed the vascular system to be filled and honeycombed by the bacteria.

Sections of Ricinus stems were cut and stained for location of the bacteria and disintegration of the tissues, which, corresponding to the slow progress of the disease in our inoculated plants, was not very extensive and not unlike figures already published by the senior writer for other plants attacked by *Bacterium solanacearum.*

Since good figures of the appearance of *Bacterium solanacearum* on agar poured plates are not very numerous, some colonies are shown enlarged 10 times (Pl. 67, B). The surface colonies on nutrient agar poured plates are irregularly roundish, white and shining by reflected light, and very fluid, so that they flow readily when placed in a vertical position. By transmitted light the colonies of this organism are brownish. By oblique light they are opalescent, the play of mother of pearl colors being usually very conspicuous. The organism is strongly aerobic, as is shown by the small buried colonies on the poured plates, feeble growth in the depths of stab cultures, and in various other ways already described. The Ricinus organism when grown on cylinders of sterile steamed potato produced exactly the same brown stain as *Bact. solanacearum* from other plants, and its reaction in milk and litmus milk is also the same.

The profound dwarfing of Ricinus plants was obtained again in 1920 with *Bacterium solanacearum* plated from the wilted North Carolina tobacco. In 1920 fuchsias were also shown to be susceptible.

[1] STANFORD, E. E., and WOLF, F. A. STUDIES ON BACTERIUM SOLANACEARUM. *In* Phytopathology, v. 7, no. 3, p. 155–165, 1 fig. Literature cited, p. 165.

PLATE 55

A.—Ricinus plants in Dothan, Ala.: *a*, dwarfed; *b*, wilting,: *c*, healthy. Photographed August, 1918.

B. Fruiting Ricinus plant, badly wilted, at Tampa, Fla.

B
A
a
b
c

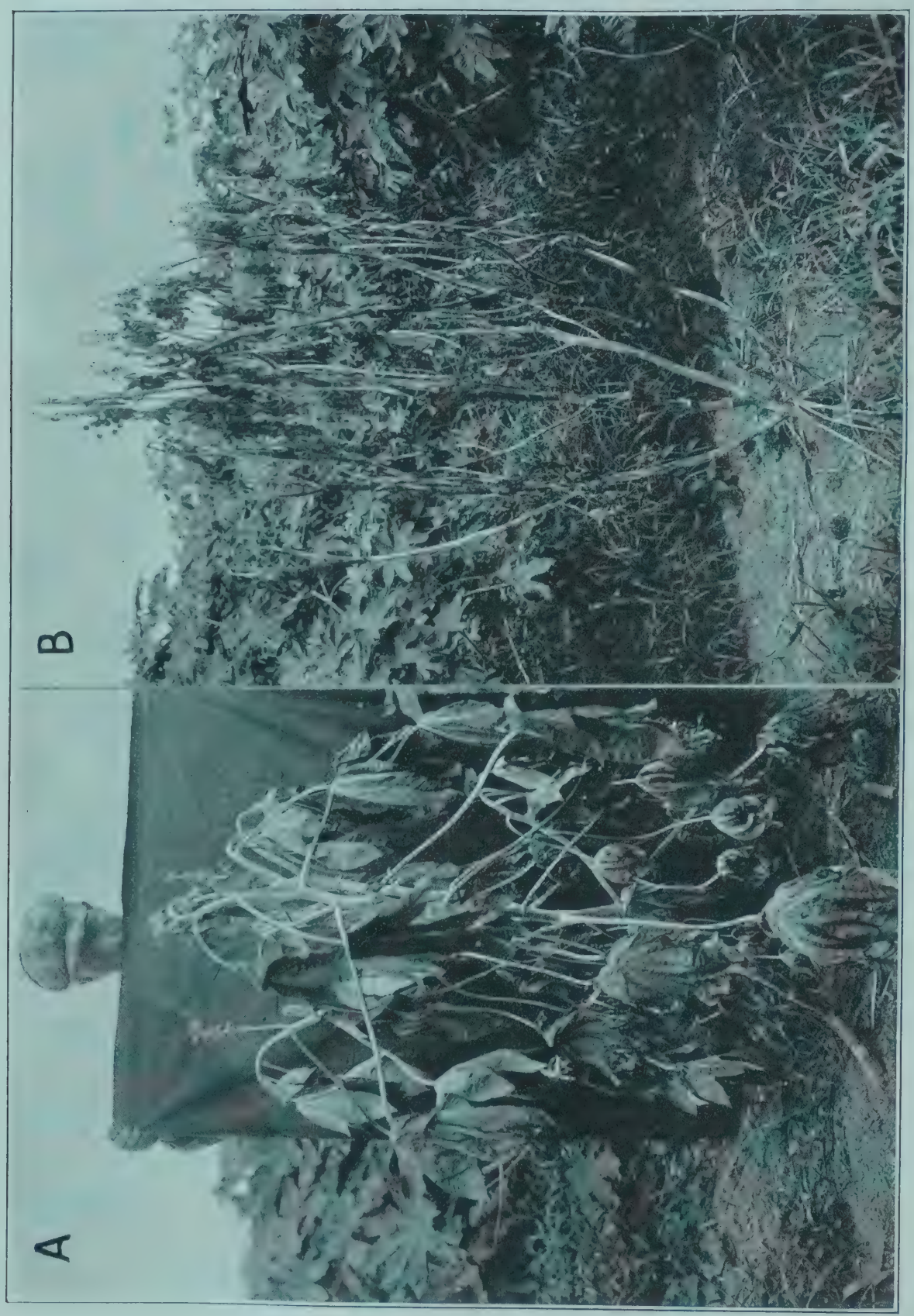
B
A

PLATE 56

A.—Root disease of Ricinus at Miami, Fla., not due to *Bacterium solanacearum*. Water table near surface.

B.—Later ttage of the same disease as in A. Healthy Ricinus plants in the background.

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PLATE 57

Effect of Ricinus wilt organism when inoculated by needle pricks at *XX* into shoots of tomato, June 26, with subculture of a colony isolated from wilted tomato No. 1, which was inoculated from subculture, of a poured-plate colony out of a wilted Ricinus plant received from Townsend, Ga. Photographed July 2, 1918. Much reduced.

X
X

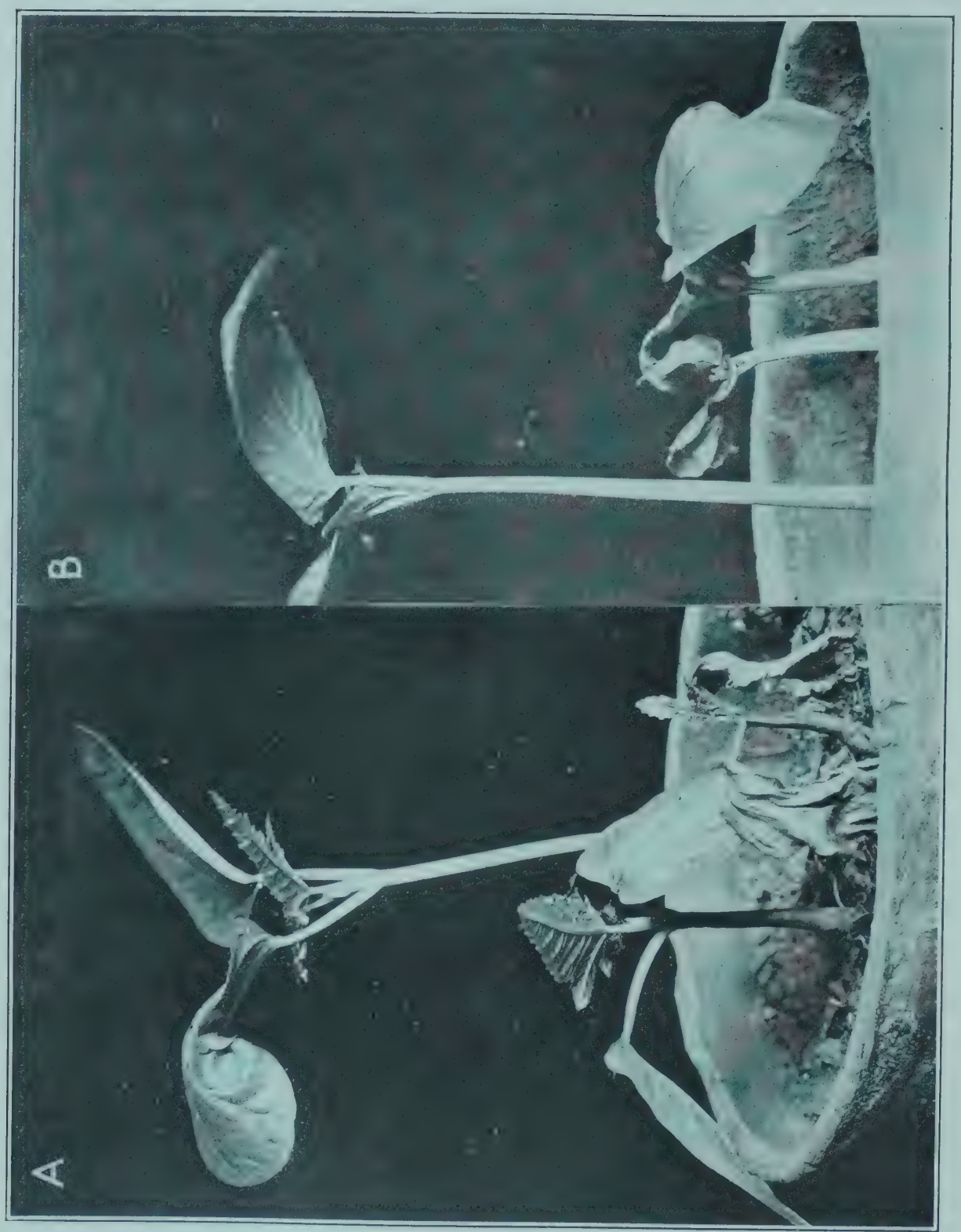

B
A

PLATE 58

A, B.—First four seedling plants to become diseased when inoculated with the Ricinus parasite. Normal control plants in the background. All the inoculated plants are badly dwarfed, but one is not yet wilting. All were inoculated June 26 by needle pricks on the base of the hypocotyl just as the seedling was emerging from the soil. Photographed July 8, 1918. In B the bacteria had penetrated into the vessels of the tap root 2½ inches from the point of inoculation.

PLATE 59

A.—First to wilt of 12 Ricinus plants inoculated July 2 at *X X* with 24-hour potato subculture from inoculated tomato No. 1. This plant when photographed was 6 inches high, while the control plants were 18 inches high. The other 11 inoculated plants were as badly dwarfed as this one, and all of them finally developed wilt. Bacteria were abundant in the vessels of the stem. Photographed July 25, 1918, nearly natural size.

B.—Cross section of middle of stem in an inoculated sunflower, showing the bacterial ooze from the vascular system. × 5.

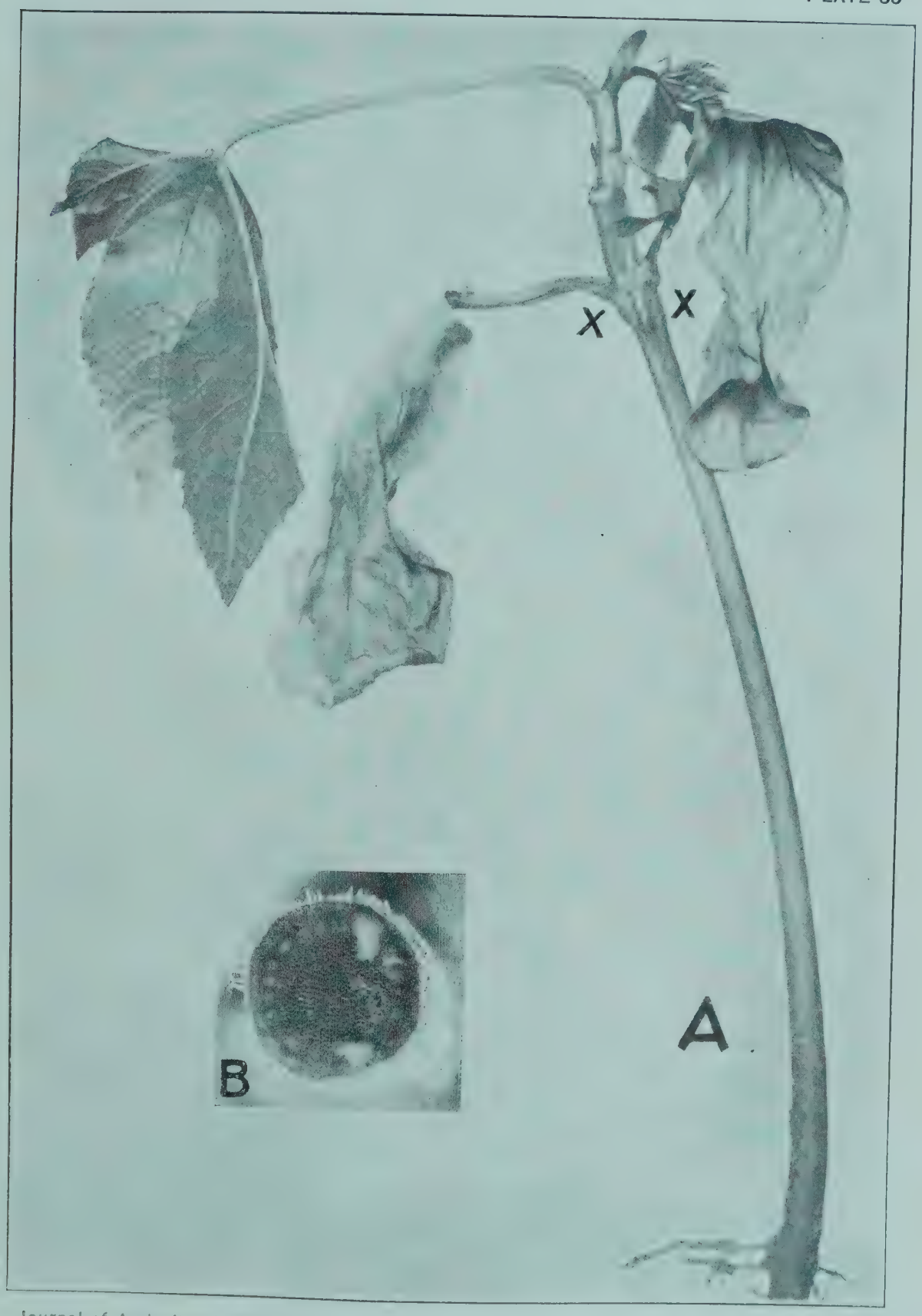

X
X
B
A

PLATE 60

Two plants showing Ricinus wilt, with two controls of the same age in the background. The wilting plants were inoculated July 2 by needle pricks in the top of the hypocotyl with 24-hour potato subculture from poured-plate colony from tomato No. 1. Introduced to show the profound dwarfing. The diseased plants were 7 inches tall and the controls 22 inches. Photographed July 30, 1918.

PLATE 61

First case of wilt in the top of a large blossoming Ricinus plant. This was inoculated by needle pricks at *X X*, on the stem and petiole, June 28, with a culture isolated from tomato No. 1. Bacteria had penetrated into all the main ribs of the blade of the wilted leaf. The brown stain and bacterial occupation of the bundles of the petiole were also very conspicuous. Photographed July 13, 1918.

X
X

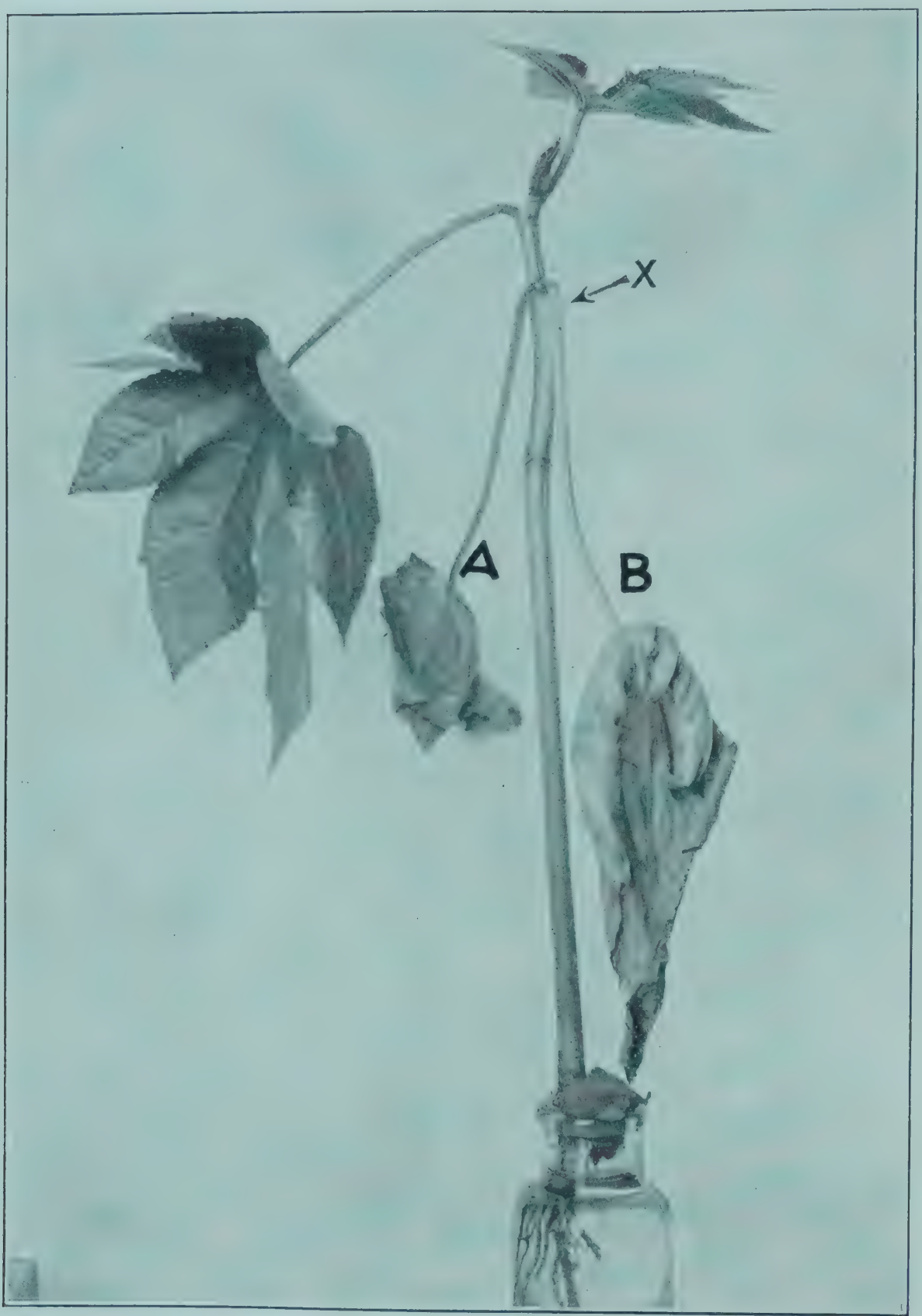

X
A
B

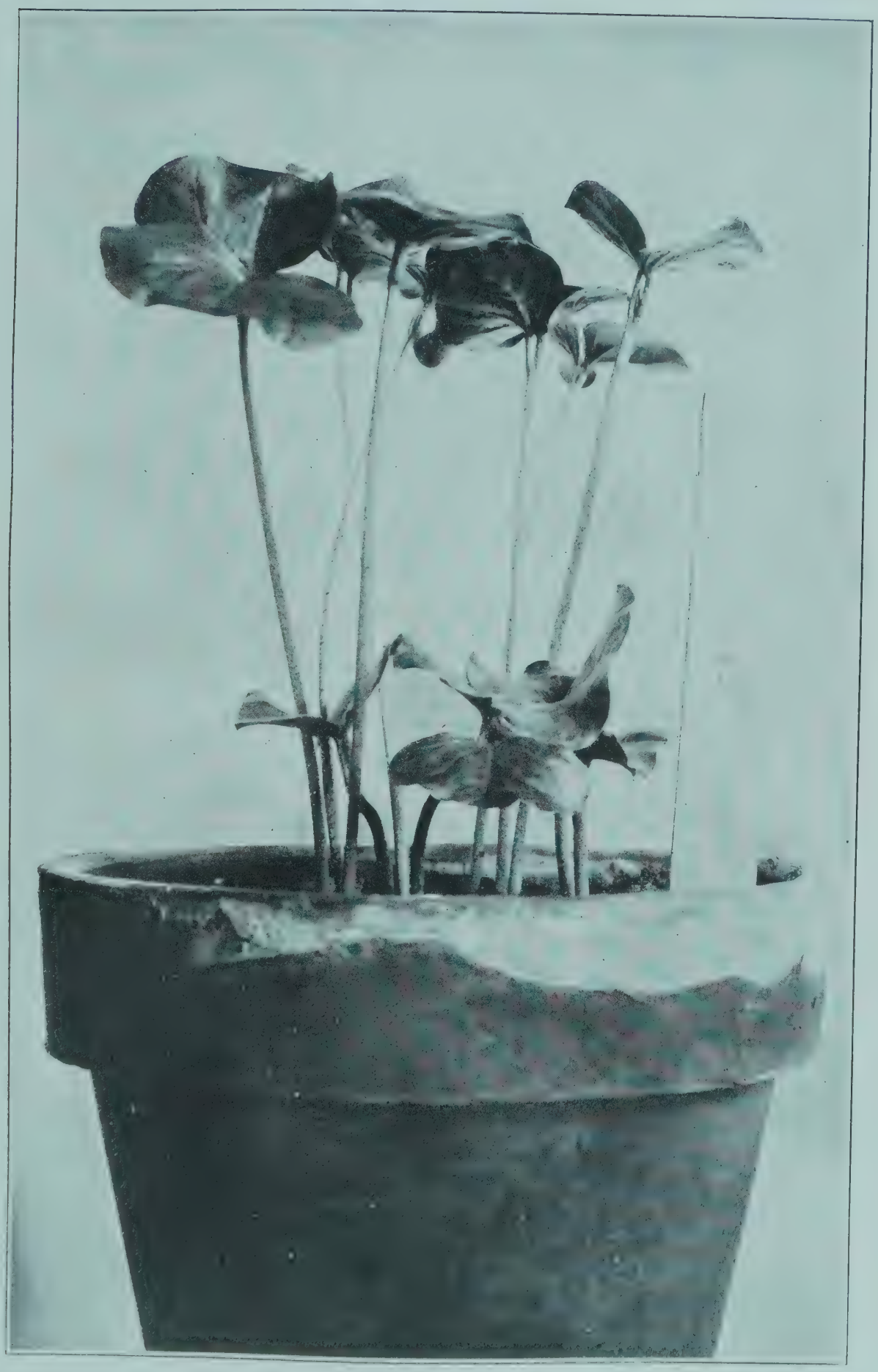
PLATE 62

Plant showing Ricinus wilt infection from seven broken roots. This was one of the control plants shown in figure 127 of "An Introduction to Bacterial Diseases of Plants."[1] The inoculated dwarfed plant was cut and buried in the soil July 17, at which time the roots of the experimental plant were broken. When the plant was photographed the bacteria were most abundant in the roots but were present in the stem as far up as *X*. They were not in the three reflexed petioles, nor in the unbroken roots. Leaves A and B became reflexed before they wilted. Reflexing of tomato petioles in this disease is very common. Photographed July 29. × ½.

[1] Smith, Erwin F. AN INTRODUCTION TO BACTERIAL DISEASES OF PLANTS. XXX, 688 pages, illus. Philadelphia and London. 1920.

PLATE 63

Dwarfing effect of *Bacterium solanacearum* when inoculated into hypocotyls of seedling cotton, variety Egyptian. Controls in the background. The inoculation was by needle pricks, and the plants were all of the same size at that time. The organism was obtained from North Carolina tobacco. A few days later all the inoculated plants wilted and shriveled, at which time they were full of the parasite. Older plants did not take the disease. Photographed July 26, 1920. Time from inoculation, 5 days. For later stages of the disease obtained with the Ricinus wilt organism see Plate 64.



A
B

PLATE 64

Ricinus wilt on seedling cotton. Controls at left. Plants inoculated in the hypocotyl by needle pricks, July 2, 1918, from inoculated tomato No. 1. Plants badly dwarfed and bacteria very abundant in the tissues. Photographed (A) July 15, 1918; (B) July 13, 1918. Each was inoculated with subculture from a poured-plate colony, and the controls were of the same size as the others when the inoculations were made.

PLATE 65

Ricinus wilt organism on *Vanilla planifolia*, inoculated on June 28, 1918, with subculture from colony 2, plate 4, June 19, out of tomato No. 1, the inoculum for which came from the Ricinus received from Townsend, Ga. Photographed July 6, 1918.

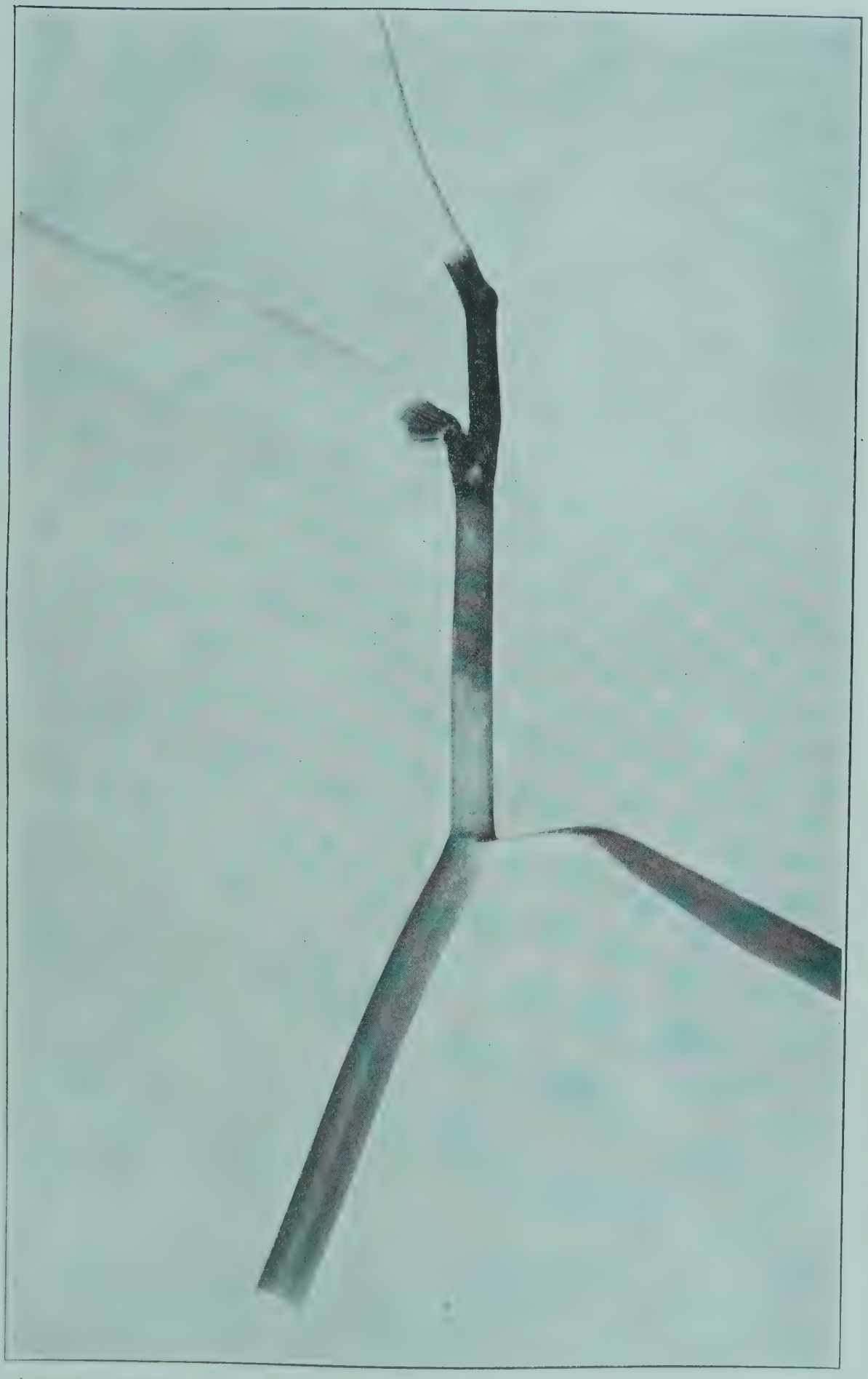


PLATE 66

Another younger tomato plant showing effect of the Ricinus parasite when inoculated by needle pricks. Control plant on right. The plant on the left (dead to the ground on August 7) was inoculated July 24 with a subculture from colony No. 1, plated from inoculated wilting sunflower No. 1. Dwarfing conspicuous; roots present on the stem. Photographed July 30, 1918. × ¼.

PLATE 67

A.—Two stems of the common garden balsam (*Impatiens balsamina*), showing internal brown striping due to the Ricinus wilt organism. The plants were inoculated June 26, 1918, and the photograph was made July 11. The bacteria were extremely abundant in the vessels of these stems. Surface unbroken.

B.—Agar poured-plate colonies of Ricinus wilt orgnism, photographed vertically and introduced to show fluid nature of the colonies and also the strongly aerobic nature of the organism, as may be seen by the small size of the buried colonies as contrasted with the two surface colonies. The colonies flow when tilted, are smooth and glistening on the surface, white by reflected light, pale brown by direct transmitted light, and opalescent by oblique light. Plate 4 days old at 26° C. It makes no difference in fluidity of the colonies whether Witte's peptone is used in the agar or Difco peptone. The opalescence by oblique light is usually very striking. Photographed June 17, 1918. × 10.

A
B

STEWART'S DISEASE OF CORN

By FREDERICK V. RAND, *Pathologist*, and LILLIAN C. CASH, *Scientific Assistant*, *Laboratory of Plant Pathology, Bureau of Plant Industry, United States Department of Agriculture*

The authors have found Stewart's disease (wilt) of corn in Georgia, South Carolina, Tennessee, Virginia, Kentucky, Missouri, Iowa, Illinois, Indiana, Ohio, Pennsylvania, District of Columbia, Maryland, Delaware, New Jersey, southern New York, and Connecticut. Although in the field the number of diseased plants has usually been under 20 per cent, as high as 100 per cent infection has sometimes been found among the earlier varieties. Cultures of the causal organism (*Aplanobacter stewarti* [E F S] McCul.) have been obtained from most of the localities where the disease has been observed by the writers. The disease was not found in Minnesota, Wisconsin, Michigan, northern New York, Vermont, New Hampshire, and Maine. However, it has been reported by other pathologists from Massachusetts, West Virginia, Michigan, Oklahoma, New Mexico, and California.

Seed of 53 varieties of sweetcorn purchased in the open market has been planted during three different seasons in Maryland. As one of the results of this field study it has been found that an arrangement of varieties according to time of maturity coincides almost exactly with an arrangement according to percentage of wilt development. The later varieties such as Zig Zag Evergreen, Stowell's Evergreen, and Country Gentleman have consistently given a very low percentage of the disease (average below 10 per cent), while the earliest varieties under the same conditions have shown a serious loss from wilt (average 25 to 57 per cent). In general, wilt prevalence among midseason varieties has been between these two extremes. First of All has shown the greatest injury, some plantings have given 100 per cent infected stalks, while the Cory group, Golden Bantam, and others of the earliest sorts approach it in susceptibility. The Evergreen group, as a whole, was little affected; and it is interesting to note that Bantam-Evergreen—a cross between Golden Bantam and Stowell's Evergreen—appeared to carry with it none of the susceptibility of the Bantam parent. Among 45 varieties of field corn planted during the same three seasons, 32 have at no time shown traces of wilt. A few of the dent corns have given 5 per cent or less, but it is the earlier flint sorts that have been found most susceptible. For example, Will's Gehu has given as high as 65 per cent of infected stalks; Square Flint, 40; Longfellow, 22; and King Philip, 19.

During the past two seasons six experiments relative to soil transmission have been carried out under field conditions. In some cases the

soil was inoculated with virulent tap water suspensions of the wilt bacteria; in other instances pieces of diseased stalks were thickly strewn in furrows, and the seed of susceptible varieties was planted among and directly over them. No evidence whatever of infection from the soil or from proximity to diseased stalks has thus far been obtained. However, the organism has been isolated from the endosperm of seeds developed on diseased plants. Furthermore, seed collected from known diseased plants gave a higher percentage of wilt than seed of the same varieties purchased in the open market.

Infection of the young corn plant from the seed was found to be largely dependent upon the growth condition of the seedling during the first week or two after planting, as influenced by soil moisture, soil texture and fertility, and temperature. Data relative to seven different plantings during three seasons (1918–1920) in our experimental fields in Maryland and two plantings during 1920 in Maine have shown that the most important single factor predisposing to infection from the seed is soil moisture. Whenever rains have been plentiful about the time of sowing the seed, wilt has later developed in abundance, whereas the same lots of seed planted during a dry period have invariably given much less infection. With moisture conditions approximately the same, the later plantings at higher temperatures have given a greater amount of wilt. Similar relations to temperature were found with inoculations in greenhouse compartments held at different temperatures. Both plantings in Maine, under conditions of drouth and low temperature, gave no wilt at all. On the other hand, the same lots of seed planted at three different dates in Maryland under more favorable conditions of both moisture and temperature gave an abundance of the disease. One planting in Maryland during the same season, although at more favorable temperatures, was sown during a period of drouth, and in this case only 2 per cent of infection occurred. At the same place during 1918 in a 4-acre variety test planted in light sandy soil during an exceptionally dry period only 10 cases of wilt occurred during the whole season. It was further noted that when seed from a single lot was planted simultaneously in light soil, in fertile sandy soil, and in rich sandy loam a considerably higher percentage of infected plants developed in the richer soil. It thus seems apparent that anything which retards the germination and early development of the seedling lessens the chances of infection from the seed. Of these environmental factors soil moisture and temperature seem to be of greatest importance.

Control methods are still in the experimental stage. However, these preliminary results seem to indicate that northern-grown seed is less likely to carry infection than that grown farther south, and that infected seed may be rendered safe for planting by a dry heat "pasteurization" at 60° to 70° C. for one hour.

QUALITY OF IRRIGATION WATER IN RELATION TO LAND RECLAMATION

By CARL S. SCOFIELD, *Agriculturist in Charge*, and FRANK B. HEADLEY, *Agriculturist*, *Office of Western Irrigation Agriculture, Bureau of Plant Industry, United States Department of Agriculture*

The so-called alkali problem on irrigated lands has two distinct phases. In one case the difficulty is due to the fact that, because of inadequate drainage or because of excessive quantities of salt in the irrigation water, the salt content of the soil solution comes to exceed the limit of tolerance of crop plants. In the other case the soil becomes relatively impermeable to water, difficult to work into good tilth after wetting, and in some cases nearly or quite unproductive. The present paper deals with this second phase of the alkali problem.

These impermeable soils or hard lands are of wide occurrence in the irrigated regions. The condition may be found in desert soils that have not been irrigated, or it may develop after a period of irrigation. It is of very common though not universal occurrence in lands that have been swamped through the rise of the ground water and later reclaimed by drainage. The condition of impermeability or hardness is one of degree, some lands though hard being much more permeable than others. It is rather uncommon to find land that is completely impermeable to irrigation water.

These hard lands sometimes contain soluble salts in excess of the generally recognized limits for crop production, but more often this is not the case. Very salty land may take water well and be soft or even "puffy" on the surface when dry, but when reclaimed by drainage and irrigation it may become so hard as to be very difficult to irrigate and difficult to work into good tilth when dry.

The subject of the slow permeability of soils has been extensively investigated, both in this country and in Europe. The condition is not confined to desert or irrigated land but is found also in many regions where the rainfall is abundant. In the latter case it is manifested either by apparent infertility or by surface erosion of the soil or by both conditions. It is a matter of common observation that the erosion of soil by rainfall is more directly associated with conditions of slow permeability to water than with conditions of topography. In other words, with a given slope a soil that is readily permeable to water is much less subject to erosion by rainfall than a soil that takes water slowly.

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Where this problem has been investigated on desert or irrigated land it has been the consensus of opinion that the condition is due to, or is at least associated with, the occurrence of sodium carbonate or "black alkali" in the soil solution. As a result of the present investigation we have reached the conclusion that the trouble is not due to sodium carbonate alone but that it may be induced by the so-called neutral salts of sodium as well.

It is well known that if a soil containing clay is leached or irrigated with water containing sodium carbonate, even in very dilute solution, the rate of percolation will become greatly retarded. Sharp [1] observed a similar retardation in the rate of percolation as a result of leaching with pure water soils to which sodium chlorid and sodium sulphate had been added. We have found that when a soil treated with sodium chlorid or sodium sulphate is leached with pure water or with solutions of these salts much more dilute than the soil solution, the rate of percolation is retarded and the percolate shows a strongly alkaline reaction. In other words, in leaching from the soil the neutral salts of sodium the permeability of the soil is reduced and the soil solution is made alkaline. These are the characteristic features of land that becomes "hard" after a period of irrigation and of salty land that has been reclaimed by drainage.

We have also observed in these investigations that this injurious effect of the salts of sodium is much reduced if the salty soil is first leached with a solution of some salt of calcium or aluminum. A soil so treated may subsequently be leached with pure water without developing the symptoms of hard land, namely, reduced permeability and an alkaline percolate. Similarly we have found that if the irrigation water contains calcium salts equal to or exceeding in quantity the salts of sodium, the soil rendered salty by such water is less likely to become impermeable when subsequently leached with pure water or with water containing these salts in the same proportion but in lower concentration.

These observations lead us to the conclusion that the quality of irrigation waters should be judged not only by considering the total quantity of the salts in solution or the proportions of the acid radicles, but also the proportion of the sodium to the calcium and magnesium. It is suggested that water, to be safe for long-continued irrigation, should be relatively rich in calcium and magnesium.

In colloquial language, waters containing relatively large quantities of calcium and magnesium are known as "hard" waters. The results of the present investigation may be summarized in the simple statement: Hard water makes soft land and soft water makes hard land.

[1] SHARP, L. T. FUNDAMENTAL INTERRELATIONSHIPS BETWEEN CERTAIN SOLUBLE SALTS AND COLLOIDS. In Univ. Calif. Pub. Agr. Sci., v. 1, no. 10, p. 291–339, 3 fig. 1916. Bibliographical footnotes.

THEORY OF THE REACTIONS

The reactions that take place in a soil that is leached with a dilute salt solution were first investigated in detail by Way.[1] Later investigators have dealt with various aspects of this subject, but too often without distinguishing clearly between the chemical and physical reactions involved. It was shown by Way that when a solution of a salt was leached through a soil containing clay a chemical reaction took place as a result of which some of the basic element of the salt was retained in the soil and an equivalent quantity of other bases was released into the soil solution and appeared in the percolate. Thus, if a solution of ammonium sulphate, sodium sulphate, or potassium sulphate was leached through a soil a portion of the base was retained by the soil and the other bases, chiefly calcium, passed into solution. It was definitely pointed out that these chemical reactions were essentially different from the physical property of soils known as adsorption which is common also to other finely divided substances such as charcoal and carbon black.

The reactions reported in the present paper appear to belong to the same category as those discovered by Way. There is, however, an additional fact to be noted. In the present investigation the question under consideration was not primarily the changes taking place in the soil solution but the changes in the physical character of a soil, both with respect to its permeability to water and to its characteristics upon drying. It has been found that leaching sodium salts through a soil causes it to become less permeable to subsequent leachings with water, and, furthermore, upon drying the soil becomes hard, a condition familiarly described in agriculture as "baking." Calcium salts, on the other hand, have been found to render the soil readily permeable for water and less likely to become hard or "baked" upon drying.

It has been shown by experiments on small samples, not here reported in detail, that the soluble salts of aluminum, such as chlorids and sulphates, are much more effective than the similar salts of calcium in improving the physical condition of impermeable soils. Soluble aluminum salts, such as aluminum sulphate, if leached through a sample even of extremely refractory soil, increases its permeability for water and completely removes the tendency to become hard on drying, the soil remaining very soft and powdery. Furthermore, these characteristics persist even after repeated leachings with distilled water.

The theory advanced to explain these facts is that the different bases in the solutions used partially displace the bases in the compounds on the surface of the soil particles. For example, when a solution of a salt of sodium is leached through a soil, the sodium will displace a portion

[1] WAY, J. Thomas. ON THE POWER OF SOILS TO ABSORB MANURE. *In* Jour. Roy. Agr. Soc. England, v. 11, p. 313–379. 1850; v. 13, p. 123–143. 1852.

[2] ——— ON THE INFLUENCE OF LIME ON THE "ABSORPTIVE PROPERTIES" OF SOILS. *In* Jour. Roy. Agr. Soc. England, v. 15, p. 491–514. 1854.

of the bases on the surface of the soil particles, itself becoming combined and remaining on the soil particles, partly at least, in the form of a silicate. This silicate, being in the form of a colloid, a hydrogel, will increase the effective size of each soil particle and thus greatly retard the movement of water through the soil and may, in fact, stop percolation entirely. Furthermore, on drying, this colloidal gel will tend to bind the soil particles in a solid mass. With solutions of calcium or aluminum, on the other hand, when the displacement of bases on the surfaces of the soil particles takes place colloid gels are not formed, and, in fact, such sodium silicate hydrogels as may exist there are almost completely eliminated or masked by the formation of practically insoluble precipitates of calcium or aluminum.

From these preliminary experiments it is also evident that the salts of aluminum, like the salts of calcium, when applied to the soil release into the soil solution other bases that may be present but not in solution. It is assumed that such bases as ammonia, sodium, potassium, calcium, and magnesium are displaced by the aluminum from their combination in the silicates of the soil and unite with the acid radical of the aluminum salt. If this assumption is correct, it would naturally follow that soluble salts of aluminum may be useful both in improving the physical condition of a soil and also in rendering other bases more soluble in the soil solution and presumably more available for the nutrition of plants.

EFFECT OF SODIUM SALTS

The injurious effects resulting from leaching a neutral sodium salt from the soil may be demonstrated by a very simple experiment. If a sample of a permeable soil be placed in a funnel over a filter paper or in an ordinary laboratory percolation tube, its percolation rate with water may be determined. If the soil is then leached with a solution of sodium chlorid or of sodium sulphate or a mixture of the two salts in constant or in increasing concentrations the rate of percolation will remain approximately constant, or it may be increased slightly over that observed with pure water and the percolate will continue clear and nearly or quite neutral in reaction. On the other hand, if the concentration of the leaching solution is reduced or if the soil is leached with pure water after treatment with the salt solutions three phenomena develop, namely, the rate of percolation is reduced, the percolate becomes turbid, and it also becomes strongly alkaline.

If the soil so treated is rich in clay, these phenomena are developed to a marked degree. With some clay soils the effect is so pronounced that it is not possible to collect a sufficient quantity of the percolate for analysis. The course of events just described is illustrated in Table I. In this case the soil used was a sample of mesa sand from the Yuma Mesa in southwestern Arizona. It was taken from a point on the mesa which had not been irrigated. The proportion of clay was very small,

and the soil was rich in calcium carbonate. In this experiment 300 gm. of dry soil were placed in the percolation tube and leached first with distilled water, 100 cc. being used for each leaching. After seven leachings with water, solutions of sodium chlorid were added, each successive solution being stronger than the preceding, until at the twentieth leaching a concentration of 5 per cent was reached. For the twenty-fifth leaching a solution of 3.7 per cent was used, and the twenty-sixth and later leachings were made with pure water.

Table I shows the concentration of the various salt solutions used, the rate of percolation, the concentration of the later percolates as shown by the electrolytic bridge, the chlorids in the percolate as determined by titration with silver nitrate and calculated as sodium chlorid,[1] the alkalinity of the percolate as determinated by titration with *N/10* sulphuric acid, using butter yellow as an indicator, and the character of the percolate with respect to turbidity.

TABLE I.—*Effect of leaching with sodium-chlorid solution followed by water on the rate of percolation and the character of the percolate*

Leaching No.	Leaching solution.	Percolation rate per minute.	Salts in percolate (by bridge).	Chlorids as NaCl.	Acid *N/10* H_2SO_4 for 100 cc. percolate.	Character of percolate.
		Cc.	*P. p. m.*	*P. p. m.*	*Cc.*	
1	Water.....	1.25	.....	58	1.80	Yellowish.
2	.....do.....	1.75	.....	23	1.40	Whitish turbid.
3	.....do.....	1.50	.....	15	.80	Do.
4	.....do.....	1.64	.....	47	.66	Do.
5	.....do.....	2.13	.....	35	.60	Do.
6	.....do.....	2.00	.....	23	.66	Do.
7	.....do.....	.....	.....	18	.66	Do.
8	NaCl p. p. m. 93...	1.54	.....	70	.66	Slightly turbid.
9	NaCl p. p. m. 210..	1.82	.....	164	.32	Clear.
10	NaCl p. p. m. 327..	.....	.....	281	.26	Do.
11	NaCl p. p. m. 421..	1.67	.....	375	.32	Do.
12	NaCl p. p. m. 515..	1.70	.....	468	.32	Do.
13	NaCl p. p. m. 795..	.....	.....	643	.34	Do.
14	NaCl p. p. m. 1,028.	1.23	.....	643	1.00	Do.
15	NaCl p. p. m. 1,987.	2.03	.....	1,286	.60	Do.
16	NaCl p. p. m. 3,226.	1.89	.....	2,457	.40	Do.
17	NaCl p. p. m. 5,150.	2.00	.....	3,510	.50	Do.
18	NaCl p. p. m. 10,520.	1.70	.....	7,030	.30	Do.
19	NaCl p. p. m. 25,000.	1.67	.....	15,780	.40	Do.
20	NaCl p. p. m. 50,000.	.....	.....	32,750	.60	Do.
21	NaCl p. p. m. 50,000.	1.43	.....	65,520	1.20	Do.
22	NaCl p. p. m. 50,000.	1.33	.....	47,969	1.30	Do.
23	NaCl p. p. m. 50,000.	1.25	.....	49,725	1.10	Do.

[1] The clear percolates, particularly those resulting from the use of the strong salt solutions, showed the presence of calcium, but the quantity was not determinated.

TABLE I.—*Effect of leaching with sodium-chlorid solution followed by water on the rate of percolation and the character of the percolate*—Continued

Leaching No.	Leaching solution.	Percolation rate per minute.	Salts in percolate (by bridge).	Chlorids as NaCl.	Acid $N/10$ H_2SO_4 for 100 cc. percolate.	Character of percolate.
		Cc.	*P. p. m.*	*P. p. m.*	*Cc.*	
24	NaCl p. p. m. 50,000.	1.00	.....	48,555	1.10	Clear.
25	NaCl p. p. m. 37,440.	.....	.....	46,780	1.20	Do.
26a	Water.....	[a].364	.....	32,770	1.40	Do.
26b	.....do.....	[b].235	.....	40,014	1.50	Very turbid.
26c	.....do.....	[c].316	1,930	562	19.20	Do.
27	.....do.....	.267	1,370	281	14.60	Do.
28	.....do.....	.....	780	199	12.40	Do.
29	.....do.....	.....	622	47	11.00	Do.
30	.....do.....	.....	569	Trace.	6.40	Turbid.
31	.....do.....	.154	506	...do....	5.90	Do.
32	.....do.....	.....	486	None.	6.70	Do.
33	.....do.....	.160	450	...do....	6.60	Do.
34	.....do.....	.142	371	...do....	4.60	Do.
35	.....do.....	.132	371	...do....	4.00	Do.
36	.....do.....	.154	350	...do....	5.60	Do.
37	.....do.....	.140	314	...do....	5.40	Do.
38	.....do.....	.131	294	...do....	4.10	Do.
39	.....do.....	.091	258	...do....	3.80	Do.
40	.....do.....	.127	233	...do....	3.04	Do.
41	.....do.....	.800	195	...do....	3.40	Do.
42	.....do.....	.760	133	...do....	2.40	Do.
43	.....do.....	.530	132	...do....	1.40	Do.

[a] 40 cc. [b] 20 cc. [c] 20 cc.

It will be observed from Table I that the percolates from the first distilled water leachings showed a slight whitish turbidity. This disappeared after the leachings with the salt solutions were begun, but the percolate became very turbid when water was again used after the salt solutions. The first distilled water leachings gave some alkalinity in the percolates; but this became less and continued very slight, with one exception, until the strongest salt solutions were used. With leaching No. 26, the first with distilled water after the salt solutions, a marked change took place. The first part of the percolate from this leaching differed little from the preceding, but the latter part was very turbid and strongly alkaline. The exception as to alkalinity noted above is seen in percolate No. 14. This may be explained by the fact that leaching No. 13 was made on Saturday and No. 14 was made on the following Monday. Although the salt solution used for No. 14 was somewhat stronger than the preceding, evaporation from the tube had been sufficient, apparently, to make the concentration of the soil solution stronger than the concentration of the leaching solution. Similar results have been noted where leachings have been made with solutions of constant strength but with intervals between the successive leachings during which the soil was allowed to dry out.

It will be noted from Table I that the alkalinity and turbidity of the percolate continued through many leachings after the use of distilled water was resumed, while the chlorids quickly disappeared. The rate of percolation also declined sharply when the leaching with distilled water was resumed.

It is probable that the course of events indicated in part in the experiment just described may be explained as a partial change of the bases in the silicates of the clay in the soil. It is assumed that some of the sodium displaces some of the calcium in these silicates and that the calcium passes out as calcium chlorid. The resultant sodium silicates remain insoluble in the presence of such strong acid radicles as the sulphates and chlorids, but as these are leached away the silicates become soluble and by their high viscosity retard the rate of percolation.

A similar substitution of bases is well known and widely used in the so-called zeolite process of water softening. In this process an artificial zeolite, rich in sodium, is used. The "hard" water to be treated is leached through the zeolite with the result that the lime is absorbed and some of the sodium is given up. From time to time the zeolite is restocked with sodium and the absorbed calcium is replaced by leaching it with a strong salt solution.

It has been shown by Kelley and Cummins [1] that such a substitution of bases takes place in the soil, presumably in the silicates, when soils are digested with dilute salt solutions. They found that calcium is extensively replaced by sodium and that sodium is replaced by calcium, though in the dilutions and with the soils with which they worked the latter reaction was much less marked than the former. The final paragraph of their conclusions is sufficiently significant to warrant quotation.

> It is suggested that the continued addition of soluble salts in the open field where the products of the reactions are removed by either the growth of crops or intermittent leaching must ultimately result in building up a chemical system different from that originally present. As will be shown later, the physical properties of the system also may be materially altered.

EFFECT OF CALCIUM SALTS

It was remarked above that the salts of calcium and of aluminum react differently in relation to subsequent leachings with distilled water than the salts of sodium. The use of gypsum (calcium sulphate) has been widely recommended as a corrective for difficulties with black alkali. These recommendations are usually based on the assumption that calcium sulphate reacts with the sodium carbonate in the soil solution to form calcium carbonate, which is very slightly soluble, and sodium sulphate, which is one of the least toxic of the sodium salts.

It is well established by numerous field experiments that gypsum exerts a beneficial effect when used on hard land. It is not very soluble,

[1] KELLEY, W. P., and CUMMINS, A. B. CHEMICAL EFFECT OF SALTS ON SOILS. *In* Soil Sci., v. 11, no. 2, p. 139–159, 7 fig. 1921. References, p. 158–159.

however, and has not been found effective where the soil is very impermeable and where it is not practicable to mix the salt thoroughly with the soil.

It has been found in these experiments that calcium chlorid is more effective in penetrating a compact and impermeable soil than the weaker solutions of calcium sulphate. It has also proved effective in making such soils readily permeable to water, and this permeability is retained after the calcium chlorid is well leached out with pure water.

In a preceding paragraph it was remarked that the injurious effects of sodium salts in irrigation water were minimized when calcium salts were also present in sufficient quantity. It may not be possible to state definitely what these proportions should be in all cases, as this may be conditioned somewhat by the character of the soil. It is clear, however, that a soil rich in lime is not proof against injury from the sodium salts carried in the irrigation water.

The neutralizing effect of calcium in solution with sodium is shown in Table II. In this experiment the soil used was also from the Yuma Mesa, but from a field that had been irrigated for 25 years with muddy water from the Colorado River, so that it was rich in silt and clay. The sample used consisted of 50 gm. of dry soil placed in a funnel over a small filter paper. It was leached each time with 100 cc. of solution or water. This soil was first leached with a strong solution of calcium chlorid which was followed by distilled water. It was then leached with a solution of equal parts of *M/10* sodium chlorid and *M/20* calcium chlorid, which was again followed by several leachings with water. It was finally leached with a solution containing 75 parts of *M/10* sodium chlorid and 25 parts *M/20* calcium chlorid, followed by distilled water.

The results given in Table II show that the percolation rate for the water leachings was little or not at all retarded when they followed the calcium chlorid or the mixture of equal parts of the chlorids of calcium and sodium. On the other hand, when the proportion of the sodium salt was much greater than that of the calcium the subsequent water leachings showed the same symptoms as when the pure sodium salt was used. In this case the symptoms were rather more pronounced than with the soil used for the leachings shown in Table I, probably because of the larger proportion of clay in the soil.

From these results it seems probable that when irrigation water contains much more sodium than calcium its use may be followed by an appreciable hardening of the soil. Where the difference in the quantity of the two salts is not great or where the total quantity of the sodium is not large it may be practicable to prevent injury by the use of gypsum on the land.

In order to test the effects of sodium and calcium salts on a soil that was known to be naturally almost impermeable, a sample was taken from a field on the Newlands project near Fallon, Nev. This soil is so hard and impermeable that it is practically unproductive, yet when it is

dry and thoroughly pulverized it has the appearance of a sandy soil. In pot cultures, when treated with gypsum and manure, it is very productive, but it has not been found possible to reclaim it completely with gypsum in the field.

The results of leaching experiments with Fallon soil are shown in Tables III and IV. In these experiments 50 gm. of dry soil were leached with 50 cc. of solution followed by the same quantity of distilled water. In one case, Table III, the chlorids of calcium and sodium were compared, and in the other case, Table IV, the sulphates of the same bases were used.

TABLE II.—*Effect of calcium chlorid and of mixtures of calcium chlorid and sodium chlorid when followed by water on the rate of percolation and the character of the percolate*

Leaching No.	Leaching solution.	Percolation rate per minute.	Acid required to neutralize 100 cc. of percolate.	Character of percolate.
		Cc.	*Cc.*	
1	*M*/6.25 $CaCl_2$	2.25	0.8	Clear.
2	Water	2.50	.6	Do.
3	do	1.58	.5	Do.
4	do	1.25	1.0	Do.
5	do	1.92	.8	Do.
6	do	1.41	.8	Do.
7	*M*/10 NaCl 50 cc. + *M*/20 $CaCl_2$ 50 cc.	1.28	.4	Do.
8	Water	2.57	.8	Do.
9	do	2.83	.8	Do.
10	do	2.66	.8	Slightly turbid.
11	do	3.00	.5	Turbid.
12	do	2.78	.7	Do.
13	*M*/10 NaCl 75 cc. + *M*/20 $CaCl_2$ 25 cc.	2.83	.3	Clear.
14	Water	2.59	.4	Turbid.
15	do	.65	.8	Very turbid.
16	do	.62	1.2	Turbid.
17	do	.036	2.8	Do.

TABLE III.—*Comparison of leaching with sodium chlorid and calcium chlorid, followed by water, showing the rate of percolation and the character of the percolate*

Leaching No.	Leaching solution.	Percolation rate per minute.	Acid required to neutralize 100 cc. of percolate.	Character of percolate.
		Cc.	*Cc.*	
1	*M*/10 NaCl	2.78	3.5	Turbid.
2	do	1.35	1.5	Clear.
3	Water	.19	8.5	Very turbid.
4	do	.023	8.5	Do.
5	do	.028	7.5	Do.
1	*M*/20 $CaCl_2$	4.50	2.0	Slightly turbid.
2	do	1.72	1.0	Clear.
3	Water	1.02	1.4	Whitish turbid.
4	do	.67	1.0	Do.
5	do	.50	3.0	Do.

TABLE IV.—*Comparison of leaching with sodium sulphate and calcium sulphate, followed by water, showing the rate of percolation and the character of the percolate*

Leaching No.	Leaching solution.	Percolation rate per minute.	Acid required to neutralize 100 cc. of percolate.	Character of percolate.
		Cc.	*Cc.*	
1	*M*/10 Na_2SO_4	3.33	3.0	Slightly turbid.
2	.....do	1.92	1.7	Clear.
3	.....do	1.92	1.5	Do.
4	Water	.62	8.5	Very turbid.
5	.....do	.007	8.5	Do.
1	$CaSO_4$, Sat. Sol	2.18	3.0	Slightly turbid.
2	.....do	1.47	1.8	Do.
3	.....do	1.16	1.8	Clear.
4	Water	1.43	1.5	Do.
5	.....do	2.17	4.0	Do.
6	.....do	1.79	2.2	Do.
7	.....do	1.73	1.5	Do.
8	.....do	1.56	1.5	Do.

Although the soil used in these experiments is almost impermeable to pure water it is easily leached with fairly strong (*M*/10) solutions of sodium chlorid or sodium sulphate. When these salt solutions are followed with water, however, the rate of percolation is at once greatly reduced and the percolate becomes turbid and alkaline. The same soil is also permeable to solutions of calcium chlorid and calcium sulphate and remains fairly permeable through the subsequent leachings with distilled water.

BASES OF IRRIGATION WATERS

In respect to the reactions produced in the soil it is assumed that potassium reacts in much the same way that sodium does and that magnesium reacts like calcium. For purposes of comparing one stream or water supply with another it seems proper to take the sum of the calcium and the magnesium as one factor and the sum of the sodium and the potassium as the other factor in a proportion. For convenience we may designate the first factor calcium and the second factor sodium and the ratio between them the calcium-sodium ratio.

The calcium-sodium ratio of the average of the analyses of 19 great rivers of the earth as given by Hilgard [1] is 84 to 16—that is, the sum of the calcium and the magnesium is to the sum of the sodium and potassium as 84 is to 16. According to the same authority the calcium-sodium ratio of the Nile is 69 to 31 for the high-water stage and 79 to 21 for the low-water stage. It may be remarked that the calcium-sodium ratio of a stream is a much more constant factor at any given point than is the total salt content.

For purposes of comparison with the ratios given above, data are assembled in Table V concerning 19 important irrigation streams in the

[1] HILGARD, E. W. SOILS . . . p. 23. New York, London, 1906.

western United States. In this table the chief bases are shown in columns 2, 3, and 4, stated in milligrams per liter. In the last column is given the calcium-sodium ratio for each stream. It will be noted that only one of the streams reported in Table V shows as high a proportion of calcium as the Nile, and none is as high as the average reported for the 19 great rivers of the earth.

In the absence of accurate data as to the extent and seriousness of the "hard" lands in the areas irrigated by these streams it is not possible to determine the correlation between the quality of the water and the condition of the land. It may be noted, however, that the land irrigated by the Belle Fourche and North Platte Rivers is notably free from difficulties as to impermeability though the soil at Belle Fourche particularly is very rich in clay. On the other hand, the land irrigated from the Salt and Gila Rivers in Arizona and that irrigated from the Truckee and Carson Rivers in Nevada is notably subject to difficulties of impermeability.

TABLE V.—*Analyses of 19 important irrigation streams in the western United States, showing quantities of calcium, magnesium, and sodium plus three-fourths of the potassium, and the proportion of the sum of the calcium and magnesium to the sum of the sodium plus three-fourths the potassium* [1]

Stream and station.	Milligrams per liter.			Proportion of the sum of the calcium and magnesium to the sum of the sodium plus three-fourths the potassium.
	Calcium.	Magnesium.	Sodium plus three-fourths of the potassium.	
Belle Fourche, Belle Fourche, S. Dak. . .	130	37	57	75 : 25
Big Horn, Fort Custer, Mont.	63	21	44	66 : 34
Boise, Boise, Idaho.	18	4	16	58 : 42
Carson, Hazen, Nev.	32	8.6	30	57 : 43
Colorado, Yuma, Ariz.	92	23	110	51 : 49
Gila, San Carlos, Ariz.	81	22	150	41 : 59
Grand, Palisade, Colo.	62	17	66	55 : 45
Green, Jensen, Utah.	55	17	45	63 : 37
Link, Klamath Falls, Oreg.	12	6	21	46 : 54
Little Colorado, Woodruff, Ariz.	68	17	110	44 : 56
Milk, Havre, Mont.	47	29	120	39 : 61
North Platte, Fort Laramie, Wyo.	69	18	40	68 : 32
Pecos, Dayton, N. Mex.	440	100	400	57 : 43
Rio Grande, El Paso, Tex.	100	18	110	52 : 48
Sacramento, Red Bluff, Calif.	16	7.4	16	59 : 41
Salt, Roosevelt, Ariz.	59	18	110	41 : 59
Shoshone, Cody, Wyo.	23	6.7	29	51 : 49
Truckee, Derby, Nev.	20	6.9	29	48 : 52
Yellowstone, Billings, Mont.	39	11	36	58 : 42

[1] STABLER, Herman. SOME STREAM WATERS OF THE WESTERN UNITED STATES. . . U. S. Geol. Survey, Water-Supply Paper 274, 188 p. 1911.

In discussing his methods of analysis, Stabler says: "The figure representing sodium and potassium together was obtained by calculating the weight of their combined chlorides to sodium. The result is in reality the amount of sodium plus three-fourths the potassium, and is so reported in the tables."

In view of the fact that the potassium content of most of these waters is low, the difference between this figure and the actual sum of sodium and potassium is probably not great.

The waters of the Colorado River and its tributaries, the Salt and the Gila, are so extensively utilized for irrigation that they merit particular emphasis in the present connection. These waters have been the subject of extensive investigation and careful analysis. The Salt River is reported in Table V, on the basis of samples taken at Roosevelt, Ariz., as showing a calcium-sodium ratio of 41 to 59. From samples collected daily for a year at a point lower down the stream, Forbes has reported in detail as shown in Table VI. The analyses made by Forbes show a higher salt content than those reported from Roosevelt and a higher proportion of sodium. It is clear that the long-continued use of water carrying such a high proportion of sodium must tend to produce impermeability in the land unless corrective measures are used.

The Gila River is characterized by great fluctuations of discharge. It drains a large watershed that is subject to torrential rains that gather into silt-laden floods. The quantity and character of the more important bases carriod by this stream are shown in Table VII, which covers the period of a year. It will be noted that the calcium-sodium ratio given for the Gila in Table V corresponds closely with that reported for the summer flood period in Table VII.

The quality of the water of the Colorado River at Yuma is given for seven characteristic periods of a year in Table VIII. It will be noted that the main river at Yuma carries less salt and a higher proportion of calcium and magnesium than its Arizona tributaries. In this it probably reflects the influence of its northern tributaries such as the Green and the Grand shown in Table V.

TABLE VI.—*Analysis of Salt River water samples taken from Arizona Canal at Mesa, Ariz., 1899–1990* [1]

Period.	Calcium plus magnesium.	Sodium plus potassium.	Calcium-sodium ratio.
	P. p. m.	*P. p. m.*	
1. High and low summer water, Aug., 1899	85.3	134.9	39 : 61
2. Summer flood water, Sept. 2 to 9, 1899	125.3	197.1	39 : 61
3. High and low summer water, Sept. 10 to Oct. 9, 1899	100.3	285.4	26 : 74
4. Low winter water, Oct. 18 to Dec. 30, 1899	68.6	325.2	21 : 79
5. Low winter water, Feb. 17 to May 30, 1900	72.9	339.3	21 : 79
6. Very low summer water, June 1 to Aug. 4, 1900	97.9	418.5	23 : 77

[1] FORBES, R. H. THE RIVER-IRRIGATING WATERS OF ARIZONA. Ariz. Agr. Exp. Sta. Bul. 44, p. 174. 1902.

TABLE VII.—*Analysis of Gila River water samples taken at head of Florence Canal, 1899–1900* [1]

Period.	Calcium plus magnesium.	Sodium plus potassium.	Calcium-sodium ratio.
	P. p. m.	*P. p. m.*	
1. Low winter water, Nov. 28, 1899 to Jan. 18, 1900	78.8	329.9	24 : 76
2. Low winter water, Feb. 1 to Mar. 7, 1900	95.2	294.5	24 : 76
3. Summer flood water, Sept. 1 to 28, 1900	69.2	104.9	40 : 60
4. Summer low water, Sept. 29 to Nov. 5, 1900	118.5	286.9	29 : 71

[1] FORBES, R. H. OP. CIT., p. 192.

TABLE VIII.—*Analysis of Colorado River water samples taken at Yuma, Ariz., 1900–1901* [1]

Period.	Calcium plus magnesium.	Sodium plus potassium.	Calcium-sodium ratio.
	P. p. m.	*P. p. m.*	
1. Low winter water, Jan. 10 to Mar. 26, 1900.....	106.3	200.5	35 : 65
2. Rising summer water, Mar. 27 to Apr. 31, 1900.....	68.6	174.2	28 : 72
3. High summer water, May 1 to June 29, 1900.....	53.8	65.1	45 : 55
4. Low summer water, June 30 to Aug. 26, 1900.....	57.8	89.2	39 : 61
5. Summer flood water (local), Aug. 1 to Oct. 1, 1901..	98.7	164.0	38 : 62
6. Summer flood water (Ariz.) Oct. 2 to Nov. 19, 1900..	152.5	203.0	43 : 57
7. Low winter water, Nov. 20, 1900, to Jan. 24, 1901...	119.7	171.7	41 : 59

[1] FORBES, R. H. OP. CIT., p. 205.

These data with respect to some of the more important irrigation streams of the western United States show that in general these waters carry a higher proportion of sodium than the Nile. If our present hypothesis is correct it may be found advisable to remedy this condition by supplying calcium, or some other high valent base such as aluminum, in soluble form to the land or to the irrigation water. There are marked differences in irrigated lands under the same water supply with respect to the tendency to become impermeable with continued irrigation. Further investigation may be expected to explain these differences and may point the way to correcting difficulties that are now acute.

SUMMARY

(1) On certain irrigated lands in the western United States the soils are not readily permeable to water and become hard and difficult to work into good tilth when dry.

(2) In extreme cases such soils are relatively unproductive because they do not absorb a sufficient quantity of water from periodical irrigations to supply the crop plants, particularly during hot, dry weather.

(3) Where the soil is not readily permeable to water it is sometimes difficult to wash out the excess of soluble salts that may be present in such quantities as to injure crop plants.

(4) This hardness or impermeability of the soil is believed to be due to the effect of sodium on the clay in the soil.

(5) A soil may become hard and its permeability become reduced by irrigating with water containing sodium carbonate.

(6) Similar results may follow when soils containing excessive quantities of sodium chlorid or sodium sulphate are leached with pure water.

(7) In the presence of certain other bases such as calcium or aluminum, in appreciable quantities and in soluble form, the injurious action of sodium on the clay does not take place.

(8) When irrigation water contains more sodium and potassium than calcium and magnesium there is danger that its continued use may cause the land to become hard and impermeable to water.

(9) Some of the important supplies of irrigation water in the United States carry more sodium and potassium than calcium and magnesium, and difficulties of hardness and impermeability of the soils are developing from the use of such water.

(10) It is believed that these difficulties may be remedied through the use of calcium or aluminum in soluble form applied either to the land or in the irrigation water.

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EFFECT OF RATION ON THE DEVELOPMENT OF PIGS

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I. CHEMICAL COMPOSITION OF PIGS AS INFLUENCED BY THE CHARACTER OF THE RATION

In the year 1911 a series of experiments [1] was begun at the Kansas Agricultural Experiment Station to study the deficiencies of corn as an exclusive ration for young growing pigs. The material for this and the following sections has been selected from the mass of data obtained in these experiments. The influence of the character of the ration upon the development of pigs has been studied by a number of investigators.[2] Forbes (*3*) noted that the specific effects of corn alone are in general a retarded development of the protein and bony tissue and an overdevelopment of fatty tissue. In a later publication by the same author and associates (*4*) the statement is made that rations deficient in constituents needed for growth resulted in a restricted development.

Sanborn (*8, 9, 10*), one of the first investigators in this country to study the effect of corn on the development of pigs, noted that pigs receiving corn only were deficient in muscular development and were overfat. Pigs receiving a higher protein ration gained weight more rapidly and with a smaller amount of feed.

Henry (*5, p. 83*) noted that the corn-fed hogs had an excessive development of fat but an inferior development of muscular tissue.

Emmett and associates (*2*) fed three lots of four 51-pound pigs during a 174-day period a ration of ground corn, blood meal, and calcium phosphate. Lot 1 was fed 0.32 pound of digestible protein per day per 100 pounds live weight; lot 2, 0.7 pound of digestible protein; lot 3, 0.94 pound of digestible protein. The slaughter test made at the end of the experiment showed that pigs in lot 1 were not normally developed. The protein intake was on too low a plane. Pigs in lot 2 showed a normal development, and there was little difference between the pigs

[1] These experiments were originated by Dr. Henry Jackson Waters while he was president of the Kansas State Agricultural College and were prosecuted under his direction by the Departments of Animal Husbandry and Chemistry of the Kansas Agricultural Experiment Station.

[2] No attempt is here made at a complete review of the literature on this subject. Bibliographies relative to this subject are given in Bulletin 169 (*11*) [3], Illinois Agricultural Experiment Station.

[3] Reference is made by number (italic) to "Literature cited," p. 341.

in lots 2 and 3, showing that the larger amount of protein given lot 3 had but little apparent influence.

Continuing the studies on the three lots of pigs mentioned above with reference to the ash content of these pigs, the authors (*11*) found that variations in the amount of digestible protein consumed did not influence significantly the percentages of distribution of total and water-soluble ash. In further studies on these same lots of pigs, Joseph (7) reports that variations in the amounts of protein consumed by growing pigs do not seem to affect the nature of the nitrogenous material produced during growth. When the supply of body protein is deficient, either quantitatively or qualitatively, it seems that only the amount of body protein is affected, while the character of the body proteins formed in the tissues remains unchanged.

The experiments conducted at the Kansas Agricultural Experiment Station cover a wide range of feeds and extended over a period of six years. Each numbered experiment refers to a year's work. For this reason the data obtained in regard to the effect of the character of the ration on the chemical composition of pigs are large in amount and varied in character.

PLAN OF THE EXPERIMENTS

The general plan of these experiments was to feed one lot of young growing pigs on corn alone, another lot on corn and ash, and other lots on corn supplemented with feeds calculated to supply either the protein or ash deficiency in corn, or both such deficiencies. The feeds supplying this protein contained ash, except for two lots in experiment VI. Each lot of animals usually consisted of three pigs. The pigs in all the lots for any one year were of uniform age, size, and breeding. But, unfortunately, the age of the pigs was not the same in different experiments. All were Duroc-Jerseys. The pigs were fed in individual stalls, and the pens were so constructed that those in the different lots had no access to foreign material. The amount consumed by each pig was carefully controlled and recorded. There were six experiments in all, one each year. The first one was of preliminary nature, and no data from this experiment are used in this and following papers. The data are taken only from pigs on which slaughter tests were made.

DESCRIPTION OF SUPPLEMENTARY FEEDS USED

BONE ASH.—This was ordinary commercial bone ash.

SYNTHETIC ASH.—This mixture was prepared according to Mendel's formula, except that magnesium citrate was omitted and calcium carbonate added.

Calcium phosphate (tertiary)....	10 parts;	sodium citrate....	15 parts.
Potassium phosphate (secondary).	37 parts;	sodium tartrate...	8 parts.
Sodium chlorid.....	20 parts;	ferric citrate.....	2 parts.
Calcium carbonate.....	92 parts.		

BLACK BLOOD ALBUMEN.—This was a commercial product containing 80.75 per cent protein and 3.61 per cent ash.

MILK PROTEIN.—This contained both the casein from skim milk separated by precipitation with acetic acid and the milk albumin separated by acetic acid and heat. It contained practically all the protein of the milk.

MILK CASEIN AND MILK ALBUMIN were the same as above, except that they were kept separate and were fed to different lots. In experiment VI commercial buttermilk casein was used.

PROTEIN-FREE SKIM MILK was the filtrate obtained after the separation of the casein and the albumin. This contained, as an average of several analyses, 0.68 per cent ash, 0.34 per cent nitrogenous compounds (N×6.25), and 5.90 per cent sugar.

CORN GERM was a commercial product containing on the average 16.50 per cent protein, 5.64 per cent ash, 19.13 per cent ether extract.

THE STARCH used was ordinary commercial cornstarch.

THE ASH-FREE BLOOD PROTEIN was prepared from blood defibrinated and chilled at the packing house. When received at the laboratory it was diluted with four or five times its volume of distilled water, acidified with acetic acid, and heated to boiling with live steam. The coagulum was filtered on linen cloth suspended on a steam cheese vat. After draining, it was washed with a large volume of distilled water and again filtered on the linen cloth and then dried over electric plates in a current of air. When dry it was ground to pass a millimeter sieve. As thus prepared it contained 95.06 per cent protein, 1.09 per cent ash, 0.0126 per cent calcium, and 0.082 per cent phosphorus. This is designated as ash-free blood protein.

METHOD OF COMBINING FEEDS

Ash as a sole supplement to corn was fed in such amounts as to make 2 or 2.5 per cent of the ration. When ash was fed in addition to some protein-supplying feed in experiment VI it constituted 4 per cent of the ration. The protein-free skim milk obtained from 3 pounds of milk was fed for each pound of corn. This proportion is designated as 1 to 3. The casein obtained from 3 pounds of milk was fed for each pound of corn to lot 16 in experiment VI, lot 22 in experiment V, and an equivalent amount to lot 35 in experiment VI. These amounts are designated casein 1 to 3. Lot 36, experiment VI, was fed casein in half these amounts. The amount is designated casein 1 to 1½. Lot 37, experiment VI, was started with the same ratio as the lots mentioned above, 1 to 3, and then the amount of casein was gradually reduced so as to give a progressively wider nutritive ratio. This is designated as casein reducing. The corn, starch, casein, and ash fed lot 34, experiment VI, were combined in such proportions as to give the nutritive ratio of average

corn. The seventh day feeding means that on every seventh day milk protein or milk casein from 3 pounds of milk was fed for each pound of corn and on six days corn alone was fed. The ash-free blood protein was fed in such amounts as to give a nutritive ratio approximating 1 to 4.

The black blood albumen was fed in such amounts as to make a nutritive ratio very nearly 1 to 5. Alfalfa pasture was the sole feed of the pigs in two lots. One representative pig from each of the latter lots was slaughtered when taken off the alfalfa pasture. After being taken off the pasture the pigs were fed corn alone. Milk albumin from 3 pounds of milk for each pound of corn was fed to lot 17 in experiment IV. In experiment V, lot 26, the albumin was fed in such amounts as to furnish an amount of protein nearly equivalent to the casein in 3 pounds of milk for each pound of corn. The former is designated as small amounts and the latter as large amounts. The corn germ was fed in the proportion of 1 pound of germ to 2 pounds of corn. The corn was fed in the form of meal, and the supplementary feeds were mixed with this meal.

AMOUNTS CONSUMED

The amounts of the several chemical constituents in the various feeds consumed by the different pigs during these trials are given in Table I. It should be noted that the data refer to only one pig from each lot.

TABLE I.—*Pounds of nutrients from corn and supplementary feeds consumed by pigs during the experiments*

Experiment No.	Lot No.	Ration.	Dry matter.	Ash.	Protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
II....	1	Corn.....	441.07	7.51	42.58	9.03	363.34	18.61
II....	2	....do.....	373.68	6.36	36.07	7.65	307.83	15.77
		Ash (bone).....	11.11	11.11	.....	.....	.....	.....
		Total.....	384.79	17.47	36.07	7.65	307.83	15.77
II....	3	Corn.....	1,097.75	18.70	105.97	22.49	904.27	46.32
		Black-blood albumen.	139.08	5.79	129.21	.....	.....	3.78
		Total.....	1,236.83	24.49	235.48	22.49	904.27	50.10
II....	4	Corn.....	1,224.41	20.85	118.20	25.08	1,008.61	51.67
		Black-blood albumen.	152.75	6.35	142.25	.....	.....	4.15
		Ash (bone).....	47.62	47.62	.....	.....	.....	.....
		Total.....	1,424.78	74.82	260.45	25.08	1,008.61	55.82
III...	6	Corn.....	206.73	3.47	22.47	5.10	165.94	9.75
III...	7	....do.....	132.59	2.22	14.41	3.27	106.44	6.25
		Ash (synthetic).....	2.99	2.99	.....	.....	.....	.....
		Total.....	135.58	5.21	14.41	3.27	106.44	6.25

TABLE I.—*Pounds of nutrients from corn and supplementary feeds consumed by pigs during the experiments*—Continued

Experiment No.	Lot No.	Ration.	Dry matter.	Ash.	Protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
III...	8	Corn	77.48	1.30	8.42	1.91	62.20	3.65
		Protein-free skim milk 1 to 3.	18.43	1.70	.85	.....	15.88	.....
		Total	95.91	3.00	9.27	1.91	78.08	3.65
III...	9	Corn	832.85	13.97	90.54	20.54	668.50	39.30
		Milk protein 1 to 3	98.95	6.33	89.39	.....	.....	3.23
		Total	931.80	20.30	179.93	20.54	668.50	42.53
IV...	13	Corn	383.45	8.60	41.88	9.24	310.93	12.80
IV...	14	....do	290.41	6.51	31.72	7.00	235.49	9.69
		Ash (synthetic)	6.42	6.42	.....	.....	.....	.....
		Total	296.83	12.93	31.72	7.00	235.49	9.69
IV...	15	Corn	340.37	7.63	37.17	8.20	276.01	11.36
		Protein-free skim milk 1 to 3.	65.81	6.43	3.24	.....	56.14	.....
		Total	406.18	14.06	40.41	8.20	332.15	11.36
IV...	16	Corn	775.04	17.39	84.65	18.67	628.46	25.87
		Casein 1 to 3	71.77	3.27	66.65	.....	.....	1.85
		Total	846.81	20.66	151.30	18.67	628.46	27.72
IV...	17	Corn	540.57	12.13	59.04	13.02	438.34	18.04
		Milk albumin (small amounts).	8.06	.71	6.71	.....	.....	.64
		Total	548.63	12.84	65.75	13.02	438.34	18.68
IV...	18	Corn	609.77	13.68	66.60	14.69	494.45	20.35
		Milk protein 1 to 3, every seventh day.	8.66	.43	8.00	.....	.....	.23
		Total	618.43	14.11	74.60	14.69	494.45	20.58
V....	21	Corn	239.47	3.97	27.25	5.13	193.19	9.93
V....	22	....do	581.25	9.47	65.72	12.42	469.65	23.99
		Milk casein 1 to 3	62.56	2.73	50.72	.....	7.91	1.20
		Total	643.81	12.20	116.44	12.42	477.56	25.19
V....	23	Corn	273.76	4.51	31.07	5.85	220.99	11.34
		Corn germ	150.34	6.97	21.47	8.66	95.38	17.86
		Total	424.10	11.48	52.54	14.51	316.37	29.20
V....	26	Corn	679.83	11.15	77.04	14.54	548.99	28.11
		Milk albumin (large amounts).	66.16	7.91	46.32	.....	8.38	3.55
		Total	745.99	19.06	123.36	14.54	557.37	31.66

TABLE I.—*Pounds of nutrients from corn and supplementary feeds consumed by pigs during the experiments*—Continued

Experiment No.	Lot No.	Ration.	Dry matter.	Ash.	Protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
V....	29	Corn	485.75	8.01	55.16	10.39	392.07	20.12
		Milk casein 1 to 3, every seventh day.	6.80	.30	5.61	.....	.76	.13
		Total	492.55	8.31	60.77	10.39	392.83	20.25
III–V	(1)	Corn	1,849.70	33.45	203.73	49.95	1,487.36	75.21
		Ash (synthetic)	40.54	40.54	.....	.....	.....	.....
		Total	1,890.24	73.99	203.73	49.95	1,487.36	75.21
III–V	(1)	Corn	1,723.43	31.32	190.75	38.34	1,392.60	70.42
		Protein-free skim milk 1 to 3.	332.43	31.69	16.03	.....	284.71	.....
		Total	2,055.86	63.01	206.78	38.34	1,677.31	70.42
VI...	30	Corn	118.61	1.79	12.99	2.41	95.95	5.47
VI...	31	....do	112.83	1.70	12.36	2.29	91.28	5.20
		Ash (synthetic)	3.15	3.15	.....	.....	.....	.....
		Total	115.98	4.85	12.36	2.29	91.28	5.20
VI...	32	Corn	203.32	3.06	22.27	4.13	164.49	9.37
		Ash-free blood protein.	23.90	.27	23.61	.....	.....	.02
		Total	227.22	3.33	45.88	4.13	164.49	9.39
VI..	33	Corn	358.77	5.41	39.29	7.29	290.24	16.54
		Ash-free blood protein.	42.18	.48	41.66	.....	.....	.. .04
		Ash (synthetic)	17.78	17.78	.....	.....	.....	.....
		Total	418.73	23.67	80.95	7.29	290.24	16.58
VI...	34	Corn	226.87	3.42	24.84	4.61	183.54	10.46
		Starch	190.71	2.21	.76	.....	187.59	.15
		Casein	27.36	.80	21.85	.....	1.47	3.24
		Ash (synthetic)	12.56	12.56	.....	.....	.....	.....
		Total	457.50	18.99	47.45	4.61	372.60	13.85
VI....	35	Corn	432.40	6.52	47.35	8.78	349.81	19.94
		Casein 1 to 3	101.03	2.94	80.70	.....	5.42	11.97
		Ash (synthetic)	14.87	14.87	.....	.....	.....	.....
		Total	548.30	24.33	128.05	8.78	355.23	31.91
VI...	36	Corn	466.03	7.02	51.03	9.47	377.02	21.49
		Casein 1 to 1½	26.06	.76	20.81	.....	1.40	3.09
		Ash (synthetic)	13.73	13.73	.....	.....	.....	.....
		Total	505.82	21.51	71.84	9.47	378.42	24.58
VI...	37	Corn	484.59	7.30	53.07	9.85	392.03	22.34
		Casein reducing	32.35	.94	25.84	.....	1.74	3.83
		Ash (synthetic)	14.28	14.28	.....	.....	.....	.....
		Total	531.22	22.52	78.91	9.85	393.77	26.17

[1] Continuation hog.

SLAUGHTER TESTS

One or two representative pigs were slaughtered and analyzed at the beginning of the trial, it being assumed that the rest of the pigs used in the trial had the same average composition as these pigs which were designated as control pigs. At the close of the trial one representative pig from each of several lots was slaughtered. In this and the following sections only the data from pigs slaughtered are used. The following are the most important data which were obtained at the time of slaughter:

Live weight.
Weight of blood as obtained.
Weight of dressed carcass.
Weight of the several internal organs.
Weight of stomach, intestines, and bladder before and after removing contents.
Weight of fatty tissues, lean tissue, skin, and bones.
Chemical composition of the various portions into which the animals were divided.

After separation, these parts or portions were ground to a pulp by passing several times through a power sausage mill. The bones were first passed through a bone cutter. In the first three experiments six samples from each pig were prepared and analyzed—namely, blood, internal organs, lean tissue, fat tissue, skin, and bones. In experiments V and VI these were combined so as to make but two samples, soft tissue and bones. Since the weight of these several portions and the percentages of moisture, ash, protein, and ether extract or fat were known,[1] it was possible to calculate the pounds of these constituents present. The percentage composition was then calculated by dividing the total pounds of each of the several constituents by the live or empty weight, as the case might be. This latter refers to the weight of the animal less the weight of the contents of the stomach, intestines, and bladder. In this paper the percentages on the basis of empty weight are used.

PERCENTAGE COMPOSITION OF THE CONTROL PIGS

The percentages of moisture, ash, protein, and ether extract or fat, as well as the age and empty weight of the control pigs slaughtered in these five experiments, are given in Table II. In experiments II and III there were two control pigs slaughtered and analyzed, and the figures given are the averages of these. Unfortunately, the ages of the pigs in the different experiments are not the same. The fact that the pigs were started at an earlier age in some experiments than in others no doubt had an influence on the results.

[1] As this ether extract was nearly pure fat, this term will be used.

TABLE I.—*Pounds of nutrients from corn and supplementary feeds consumed by pigs during the experiments*—Continued

Experiment No.	Lot No.	Ration.	Dry matter.	Ash.	Protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
V....	29	Corn	485.75	8.01	55.16	10.39	392.07	20.12
		Milk casein 1 to 3, every seventh day.	6.80	.30	5.61	.....	.76	.13
		Total	492.55	8.31	60.77	10.39	392.83	20.25
III–V	(1)	Corn	1,849.70	33.45	203.73	49.95	1,487.36	75.21
		Ash (synthetic)	40.54	40.54	.....	.....	.....	.....
		Total	1,890.24	73.99	203.73	49.95	1,487.36	75.21
III–V	(1)	Corn	1,723.43	31.32	190.75	38.34	1,392.60	70.42
		Protein-free skim milk 1 to 3.	332.43	31.69	16.03	.....	284.71	.....
		Total	2,055.86	63.01	206.78	38.34	1,677.31	70.42
VI...	30	Corn	118.61	1.79	12.99	2.41	95.95	5.47
VI...	31	....do	112.83	1.70	12.36	2.29	91.28	5.20
		Ash (synthetic)	3.15	3.15	.....	.....	.....	.....
		Total	115.98	4.85	12.36	2.29	91.28	5.20
VI...	32	Corn	203.32	3.06	22.27	4.13	164.49	9.37
		Ash-free blood protein.	23.90	.27	23.61	.....	.....	.02
		Total	227.22	3.33	45.88	4.13	164.49	9.39
VI..	33	Corn	358.77	5.41	39.29	7.29	290.24	16.54
		Ash-free blood protein.	42.18	.48	41.66	.....	.....	.. .04
		Ash (synthetic)	17.78	17.78	.....	.....	.....	.....
		Total	418.73	23.67	80.95	7.29	290.24	16.58
VI...	34	Corn	226.87	3.42	24.84	4.61	183.54	10.46
		Starch	190.71	2.21	.76	.....	187.59	.15
		Casein	27.36	.80	21.85	.....	1.47	3.24
		Ash (synthetic)	12.56	12.56	.....	.....	.....	.....
		Total	457.50	18.99	47.45	4.61	372.60	13.85
VI....	35	Corn	432.40	6.52	47.35	8.78	349.81	19.94
		Casein 1 to 3	101.03	2.94	80.70	.....	5.42	11.97
		Ash (synthetic)	14.87	14.87	.....	.....	.....	.....
		Total	548.30	24.33	128.05	8.78	355.23	31.91
VI...	36	Corn	466.03	7.02	51.03	9.47	377.02	21.49
		Casein 1 to 1½	26.06	.76	20.81	.....	1.40	3.09
		Ash (synthetic)	13.73	13.73	.....	.....	.....	.....
		Total	505.82	21.51	71.84	9.47	378.42	24.58
VI...	37	Corn	484.59	7.30	53.07	9.85	392.03	22.34
		Casein reducing	32.35	.94	25.84	.....	1.74	3.83
		Ash (synthetic)	14.28	14.28	.....	.....	.....	.....
		Total	531.22	22.52	78.91	9.85	393.77	26.17

[1] Continuation hog.

SLAUGHTER TESTS

One or two representative pigs were slaughtered and analyzed at the beginning of the trial, it being assumed that the rest of the pigs used in the trial had the same average composition as these pigs which were designated as control pigs. At the close of the trial one representative pig from each of several lots was slaughtered. In this and the following sections only the data from pigs slaughtered are used. The following are the most important data which were obtained at the time of slaughter:

Live weight.
Weight of blood as obtained.
Weight of dressed carcass.
Weight of the several internal organs.
Weight of stomach, intestines, and bladder before and after removing contents.
Weight of fatty tissues, lean tissue, skin, and bones.
Chemical composition of the various portions into which the animals were divided.

After separation, these parts or portions were ground to a pulp by passing several times through a power sausage mill. The bones were first passed through a bone cutter. In the first three experiments six samples from each pig were prepared and analyzed—namely, blood, internal organs, lean tissue, fat tissue, skin, and bones. In experiments V and VI these were combined so as to make but two samples, soft tissue and bones. Since the weight of these several portions and the percentages of moisture, ash, protein, and ether extract or fat were known,[1] it was possible to calculate the pounds of these constituents present. The percentage composition was then calculated by dividing the total pounds of each of the several constituents by the live or empty weight, as the case might be. This latter refers to the weight of the animal less the weight of the contents of the stomach, intestines, and bladder. In this paper the percentages on the basis of empty weight are used.

PERCENTAGE COMPOSITION OF THE CONTROL PIGS

The percentages of moisture, ash, protein, and ether extract or fat, as well as the age and empty weight of the control pigs slaughtered in these five experiments, are given in Table II. In experiments II and III there were two control pigs slaughtered and analyzed, and the figures given are the averages of these. Unfortunately, the ages of the pigs in the different experiments are not the same. The fact that the pigs were started at an earlier age in some experiments than in others no doubt had an influence on the results.

[1] As this ether extract was nearly pure fat, this term will be used.

TABLE II.—*Chemical composition of control pigs*

Experiment No.	Age of pigs.	Empty weight.	Moisture.	Ash.	Protein.	Ether extract.
	Months.	*Pounds.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
II	4½	50.38	50.38	3.30	13.03	27.39
III	3½	37.65	49.03	2.79	11.98	29.00
IV	4	40.97	55.67	5.92	16.35	25.30
V	2	34.63	60.50	3.06	15.42	14.87
VI	2¼	21.72	57.18	2.81	13.44	15.15

From experiment III, one pig from the lot fed corn and synthetic ash and one from the lot fed corn and protein-free skim milk were continued on these feeds until they were nearly 3 years old. At the time the pigs from experiment V were slaughtered these two were included in the test. These two pigs are indicated by the name of the ration and the numerals III–V. For convenience these two are called continuation hogs, since they were continued from experiment III to the end of experiment V.

CHEMICAL COMPOSITION OF REPRESENTATIVE PIGS

The figures for percentages of moisture, ash, protein, and ether extract in representative pigs from different lots in these experiments are found in Table III. These figures were calculated on the basis of empty weight. For purposes of study these figures are arranged in the order of increasing empty weights, beginning with the smallest.

MOISTURE CONTENT

The figures for moisture percentages are plotted in figure 1, using the empty weights as ordinates and the percentages of moisture as abscissas. The roman numerals throughout the legends refer to the different experiments. On the whole, the percentage of moisture decreases as the size increases. This curve shows that these pigs can be divided roughly into three classes: (1) Below 100 pounds, (2) between 100 and 300 pounds, and (3) above 300 pounds. In pigs below 100 pounds the variations are irregular in the extreme, and all the pigs in this class had deficient rations. In the second class the variation is somewhat more regular, and there is no distinct tendency to decrease in percentage of moisture with increasing size. The pigs weighing more than 100 pounds and less than 300 pounds, which were fed a ration supplying both protein and ash in addition to corn, have a tendency to carry a higher moisture content than those which were fed either protein or ash alone as a supplement. Above 300 pounds the curve shows distinctly that the tendency is to a decrease in moisture as the size increases. Size is the predominating factor when the ration produced an animal weighing between 300 and 400 pounds. This is due to the accumulation of fat, as will be shown later.

TABLE III.—*Chemical composition of representative pigs slaughtered at the end of each experiment*

[Arranged in the order of increasing weights of pigs]

Experiment No.	Lot No.	Ration.	Age of pig at start.	Empty weight.	Moisture.	Ash.	Protein.	Ether extract.
			Months.	*Pounds.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
III	8	Corn and protein-free skim milk	3½	28.06	59.49	4.34	15.20	15.02
VI	30	Corn alone	2¼	28.60	65.36	3.81	14.30	11.40
VI	31	Corn and synthetic ash	2¼	34.37	58.11	2.59	12.08	15.51
III	7	....do	3½	45.62	46.01	3.44	11.28	23.65
IV		Alfalfa pasture	4	51.25	60.35	4.53	19.39	15.43
V	21	Corn alone	2	62.71	44.70	2.22	11.48	40.50
III	6	....do	3½	63.28	39.93	2.69	11.44	34.46
III		Alfalfa pasture	3½	63.60	66.68	3.96	16.41	5.27
VI	32	Corn and ash-free blood protein	2¼	72.18	49.65	2.09	14.11	37.26
IV	14	Corn and synthetic ash	4	86.40	45.03	2.88	12.08	40.83
IV	13	Corn alone	4	105.25	40.82	2.57	10.94	47.16
II	2	Corn and bone ash	4½	122.80	36.85	2.71	11.25	48.42
IV	15	Corn and protein-free skim milk	4	127.45	38.10	2.27	9.59	50.95
V	29	Corn and casein every seventh day	2	139.84	37.69	1.63	10.45	48.78
V	23	Corn and corn germ	2	140.44	45.88	1.83	12.03	38.37
II	1	Corn alone	4½	141.49	34.83	2.16	9.88	49.91
VI	33	Corn, ash-free blood protein, and synthetic ash	2¼	156.86	46.91	2.63	13.02	40.44
VI	34	Corn, starch, casein, and synthetic ash	2¼	159.35	43.74	2.43	12.20	38.55
IV	17	Corn and milk albumin (small amounts)	4	180.99	36.61	1.75	10.95	52.31
VI	37	Corn, casein reducing, and synthetic ash	2¼	192.20	44.86	2.61	12.30	44.20
VI	36	Corn, casein 1 to 1½, and synthetic ash	2¼	195.66	41.18	2.46	12.03	40.72
VI	35	Corn, casein 1 to 3, and synthetic ash	2¼	199.71	44.78	2.74	12.69	40.84
IV	18	Corn and milk protein every seventh day	4	209.95	36.49	1.96	10.81	51.21
V	22	Corn and casein 1 to 3	2	211.22	39.33	2.10	12.65	46.71
V	26	Corn and milk albumin (large amounts)	2	245.15	37.39	2.02	11.33	46.30
IV	16	Corn and casein 1 to 3	4	282.58	39.67	1.92	9.28	47.63
III	9	Corn and milk protein	3½	318.93	32.68	1.60	10.37	52.42
III–V	Continuation hogs.	Corn and synthetic ash	3½	364.52	30.92	1.71	8.32	56.75
III–V	....do	Corn and protein-free skim milk	3½	366.42	33.23	2.05	9.87	53.46
II	3	Corn and black blood albumen	4½	378.20	27.66	1.47	9.22	59.08
II	4	Corn, black blood albumen, and bone ash	4½	389.26	25.66	1.76	8.51	61.53

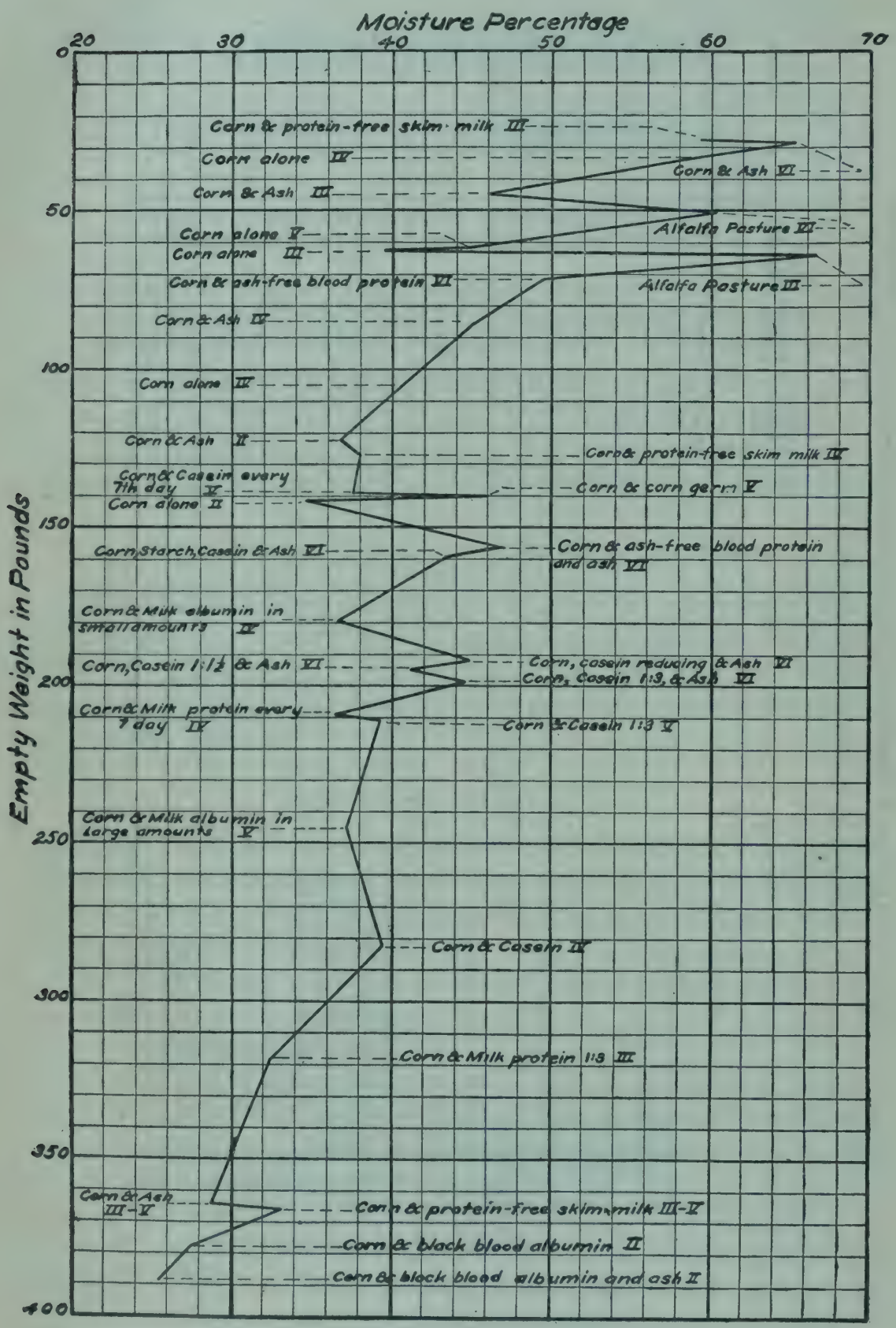

FIG. 1.—Graph showing the relation between percentage of moisture and empty weight.

FAT CONTENT

The figures for the percentages of fat are plotted in figure 2 in a manner similar to that used in figure 1. As in the case of the moisture percentages, the pigs below 100 pounds show extreme variation. Between 100 pounds and 250 pounds the variations show no distinct tendency to

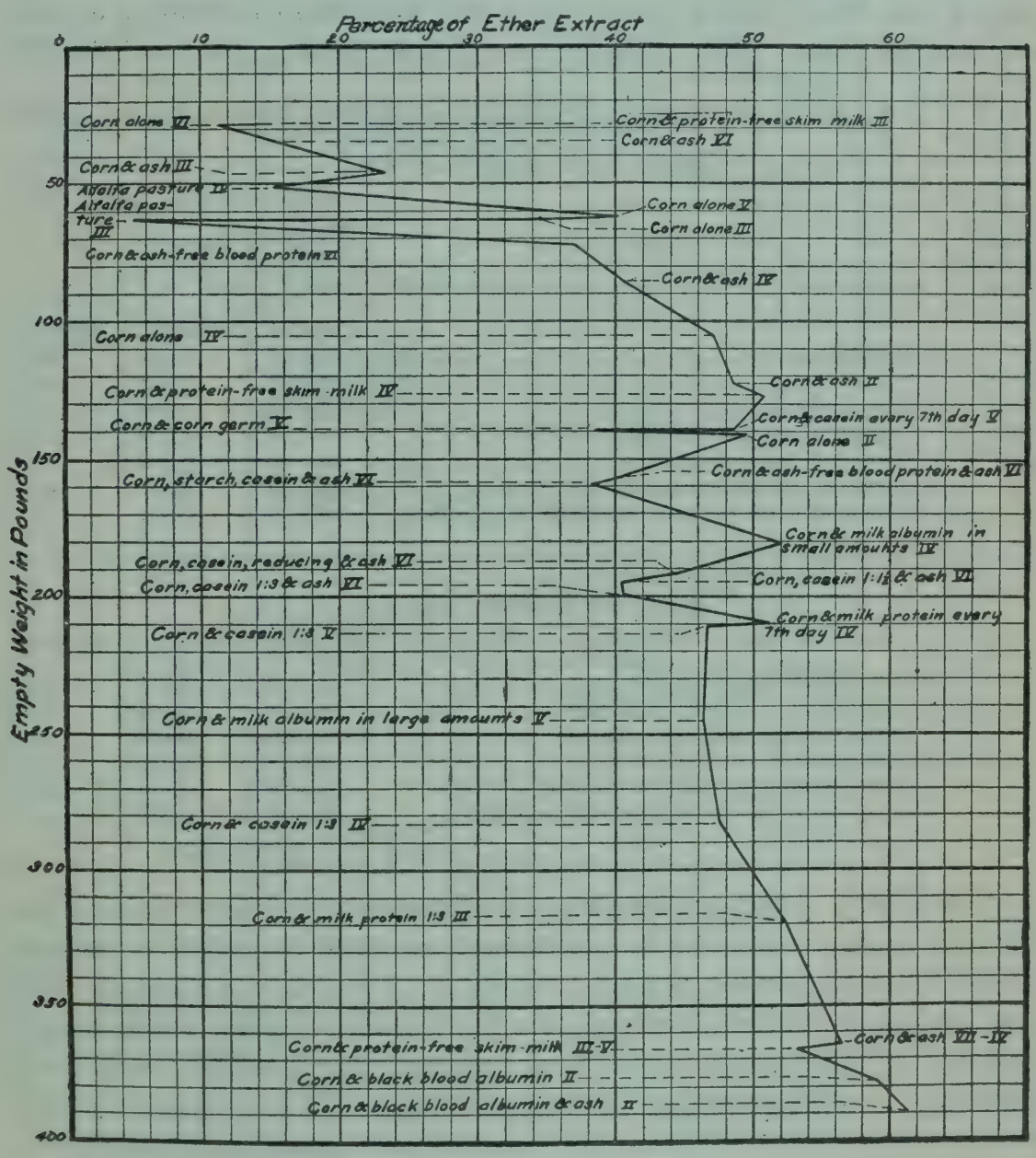

FIG. 2.—Graph showing the relation between percentage of fat (ether extract) and empty weight.

increase or decrease. Above 250 pounds the tendency to increase in fat content is pronounced.

COMPARISON OF THE PERCENTAGE OF MOISTURE AND FAT

The pigs which have a high moisture content have a low fat content. A study of the figures in Table III and the graphs in figures 1 and 2 will show that in all pigs whose weight is below 100 pounds the percentage of moisture is higher than the percentage of fat. In all pigs whose

weight is above 225 pounds the percentage of fat is higher than the percentage of moisture. Between these two limits the variation is irregular. If this irregularity is studied further it will be found that wherever the moisture content is higher than the fat content corn has been supplemented by both additional protein and ash. When the fat content is higher than the moisture content the corn has been supplemented only by one feed furnishing either additional protein or ash, or corn was fed alone. This is readily seen by the figures given in Table IV.

TABLE IV.—*Percentage of moisture and fat in pigs of between 100 and 225 pounds weight*

MOISTURE LOWER THAN FAT

Experiment No.	Ration.	Empty weight.	Moisture.	Fat.
		Pounds.	*Per cent.*	*Per cent.*
II	Corn alone	141. 50	33. 0	47. 0
IV	do	105. 25	38. 0	44. 0
II	Corn and ash	122. 80	35. 0	45. 0
IV	Corn and protein-free skim milk	127. 45	35. 0	47. 0
IV	Corn and milk albumin	181. 00	33. 0	47. 0
V	Corn and casein, seventh day feeding	139. 84	33. 0	43. 0
IV	Corn and milk protein, seventh day feeding	209. 95	34. 0	51. 0

MOISTURE HIGHER THAN FAT

V	Corn and corn germ	140. 44	40. 0	33. 0
VI	Corn, starch, casein, and ash	159. 35	42. 0	37. 0
VI	Corn, casein, and ash 1 to 1½	195. 66	40. 0	40. 0
VI	Corn, casein, and ash 1 to 3	199. 71	43. 0	40. 0
VI	Corn, casein, and ash widening	192. 20	42. 0	41. 0
VI	Corn, ash-free blood protein, and ash	156. 86	46. 0	39. 0

All rations in which corn was not supplemented by both protein and ash were either consumed in insufficient amounts or failed to produce growth when consumed in large amounts. If a ration is partially deficient, yet adequate for restricted growth, it produces a pig of abnormally high fat content. If the ration is so deficient that growth is very much stunted the moisture content is abnormally high. When pigs grew above 200 pounds there was a regular and constant increase in percentage of fat.

ASH CONTENT

The figures for ash percentages on the basis of empty weight are plotted in figure 3. In pigs weighing less than 80 pounds the variation is very irregular. The two pigs fed on alfalfa pasture and the one fed corn and protein-free skim milk had the highest percentage of ash. Among the pigs which weighed over 80 pounds and less than 210 pounds those fed corn and corn germ, corn and casein every seventh day, and corn and milk albumin in small amounts had the lowest percentage of ash. While

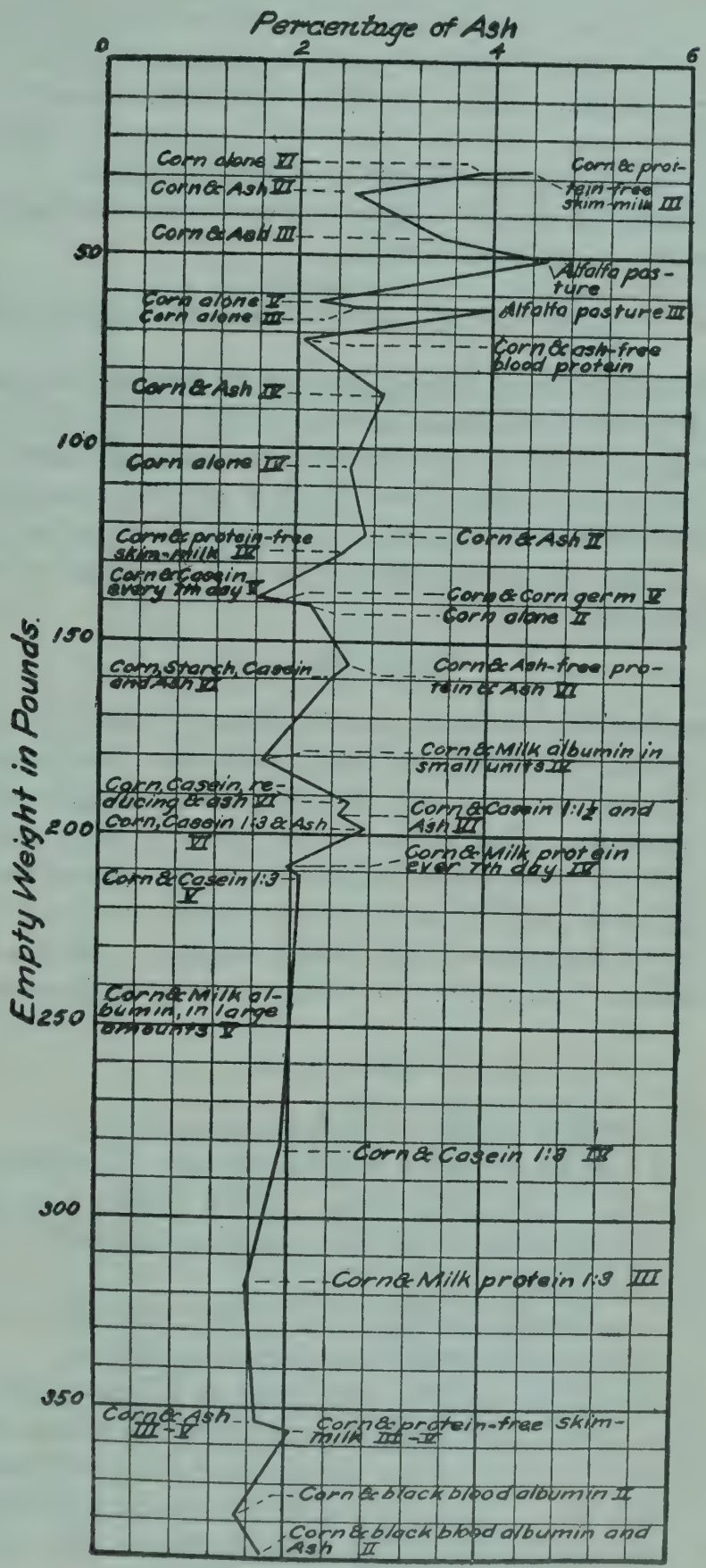
Percentage of Ash
0
2
4
6
Empty Weight in Pounds.
50
100
150
200
250
300
350
Corn alone VI
Corn & Ash III
Corn & Ash III
Corn & protein-free skim-milk III
Alfalfa pasture
Corn alone V
Corn alone III
Alfalfa pasture III
Corn & ash-free blood protein
Corn & Ash IV
Corn alone III
Corn & protein-free skim-milk III
Corn & Ash II
Corn & Casein every 7th day V
Corn & Corn germ V
Corn alone II
Corn, Starch, Casein and Ash V
Corn & Ash-free protein & Ash VI
Corn & Milk albumin in small units IV
Corn, Casein reducing & ash VI
Corn, Casein 1:3 & Ash VI
Corn & Casein 1:1½ and Ash III
Corn & Milk protein ever 7th day IV
Corn & Casein 1:3 V
Corn & Milk albumin, in large amounts V
Corn & Casein 1:3 III
Corn & Milk protein 1:3 III
Corn & Ash III-V
Corn & protein-free skim-milk III-V
Corn & black blood albumin II
Corn & black blood albumin and Ash II

FIG. 3.—Graph showing the relation between percentage of ash and empty weight.

the corn germ supplied 6.97 pounds of ash in addition to that present in the corn, yet this ash contained only 0.03 pound calcium, an inadequate amount. The casein furnished only 0.36 pound ash and the milk albumin 0.71 pound. These amounts were inadequate for the needs of the growing pig. All the pigs which attained a growth of 210 pounds or more had an abundance of ash in the ration.

Proportionately there is a smaller change in the ash percentage than in the percentage of moisture and fat.

ASH PERCENTAGES ON THE BASIS OF EMPTY WEIGHT, LESS FAT

The gradual decrease in ash percentages as the pigs increase in size is almost wholly due to the large increase in percentage of fat. In Table V the percentage of ash is calculated on the basis of empty weight less fat, or the relation of ash to the amount of body substance less fat. These results are plotted in figure 4. The general direction of this curve shows that the ash percentage tends to remain constant in the tissues of the body not fat.

PROTEIN CONTENT

The figures for percentages of protein given in Table III are plotted in figure 5. With a few exceptions, the protein curve has the same general direction as the moisture curve. As pigs increase in size the percentage of protein decreases. This is due to the relatively large increase of fat. The figures for the percentages of protein on the basis of empty weight less fat are given in Table V, and the curve is given in figure 6. The general direction of this curve is practically straight. In this respect it is similar to the curve for ash on the basis of empty weight less fat.

CONCLUSIONS

The data presented permit the following conclusions:

1. Pigs fed rations decidedly deficient in ash or protein had a very restricted growth. The composition of their bodies was characterized by wide variations among the different animals, generally a low fat and a high moisture content.

2. Pigs fed a partially deficient ration, but one which was sufficient to produce a restricted growth, had a high fat content.

3. In pigs fed a ration that was balanced in respect to the protein and ash requirements, the moisture and fat content were nearly equal, with a tendency for the fat percentage to be a little lower than the moisture percentage. In pigs whose ration was partially restricted, the fat content was higher than the moisture content. This statement applies only to pigs weighing less than 225 pounds.

TABLE V.—*Percentage of ash and protein in pigs, calculated on the basis of empty weight less fat*

[Arranged in order of increasing empty weight less fat]

Experiment No.	Lot No.	Ration.	Empty weight.	Weight of fat.	Empty weight less fat.	Weight of ash.	Weight of protein.	Percentage of ash in empty weight less fat.	Percentage of protein in empty weight less fat.
			Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*		
III	8	Corn and protein-free skim milk	28.06	4.22	23.84	1.22	4.27	5.12	17.91
VI	30	Corn alone	28.60	3.26	25.34	1.09	4.09	4.30	16.14
VI	31	Corn and synthetic ash	34.37	5.33	29.04	.89	4.15	3.07	14.29
III	7	....do	45.62	10.79	34.83	1.56	5.14	4.48	14.76
V	21	Corn alone	62.71	25.40	37.31	1.39	7.20	3.73	19.30
III	6	....do	63.28	21.80	41.48	.58	7.22	1.40	17.40
IV		Alfalfa pasture	51.25	9.26	41.99	2.72	11.64	6.48	27.73
VI	32	Corn and ash-free blood protein	72.18	26.91	45.27	1.51	10.19	3.34	22.51
IV	14	Corn and synthetic ash	86.40	34.99	51.41	2.47	10.35	4.80	20.13
IV	13	Corn alone	105.25	48.91	56.34	2.67	11.34	4.74	20.12
III		Alfalfa pasture	63.60	3.35	60.25	2.52	10.44	4.18	17.33
IV	15	Corn and protein-free skim milk	127.45	64.35	63.10	2.87	12.11	4.55	19.19
II	2	Corn and bone ash	122.80	54.48	68.32	3.06	12.67	4.48	18.54
V	29	Corn and casein every seventh day	139.84	68.21	71.63	2.28	14.61	3.18	20.40
II	1	Corn alone	141.49	64.71	76.78	2.79	12.81	3.63	16.68
V	23	Corn and corn germ	140.44	53.89	86.55	2.57	16.90	2.97	19.52
IV	17	Corn and albumin (small amounts)	180.99	93.17	87.82	3.12	19.50	3.55	22.19
VI	33	Corn, ash-free blood protein, and synthetic ash	156.86	63.43	93.43	4.11	20.43	4.40	21.86
IV	18	Corn and milk protein every seventh day	209.95	113.02	96.93	4.09	22.59	4.22	23.29
VI	34	Corn, starch, casein, and synthetic ash	159.35	61.44	97.91	3.89	19.45	3.97	19.86
VI	37	Corn, casein reducing, and synthetic ash	192.20	85.55	106.65	5.01	23.64	4.70	22.16
V	22	Corn and casein 1 to 3	211.22	98.67	112.55	4.43	26.73	3.93	23.74
VI	36	Corn, casein 1 to 1½, and synthetic ash	195.66	79.68	115.98	4.82	23.54	4.16	20.30
VI	35	Corn, casein 1 to 3, and synthetic ash	199.71	81.56	118.15	5.47	25.33	4.63	21.44
V	26	Corn and albumin (large amounts)	245.15	113.53	131.62	4.96	27.78	3.77	21.11
IV	16	Corn and casein 1 to 3	282.58	136.62	145.96	5.52	26.63	3.76	18.18
III	9	Corn and milk protein	318.93	167.22	151.71	5.09	33.08	3.36	21.81
III–V	Continuation hog.	Corn and synthetic ash	364.52	206.85	157.67	6.24	30.34	3.96	19.24
II	4	Corn, black-blood albumen, and bone ash	389.26	230.64	158.62	6.59	31.89	4.15	20.11
II	3	Corn and black-blood albumen	378.20	215.17	163.03	5.36	33.59	3.29	20.60
III–V	Continuation hog.	Corn and protein-free skim milk	366.42	195.87	170.55	7.50	36.16	4.40	21.20

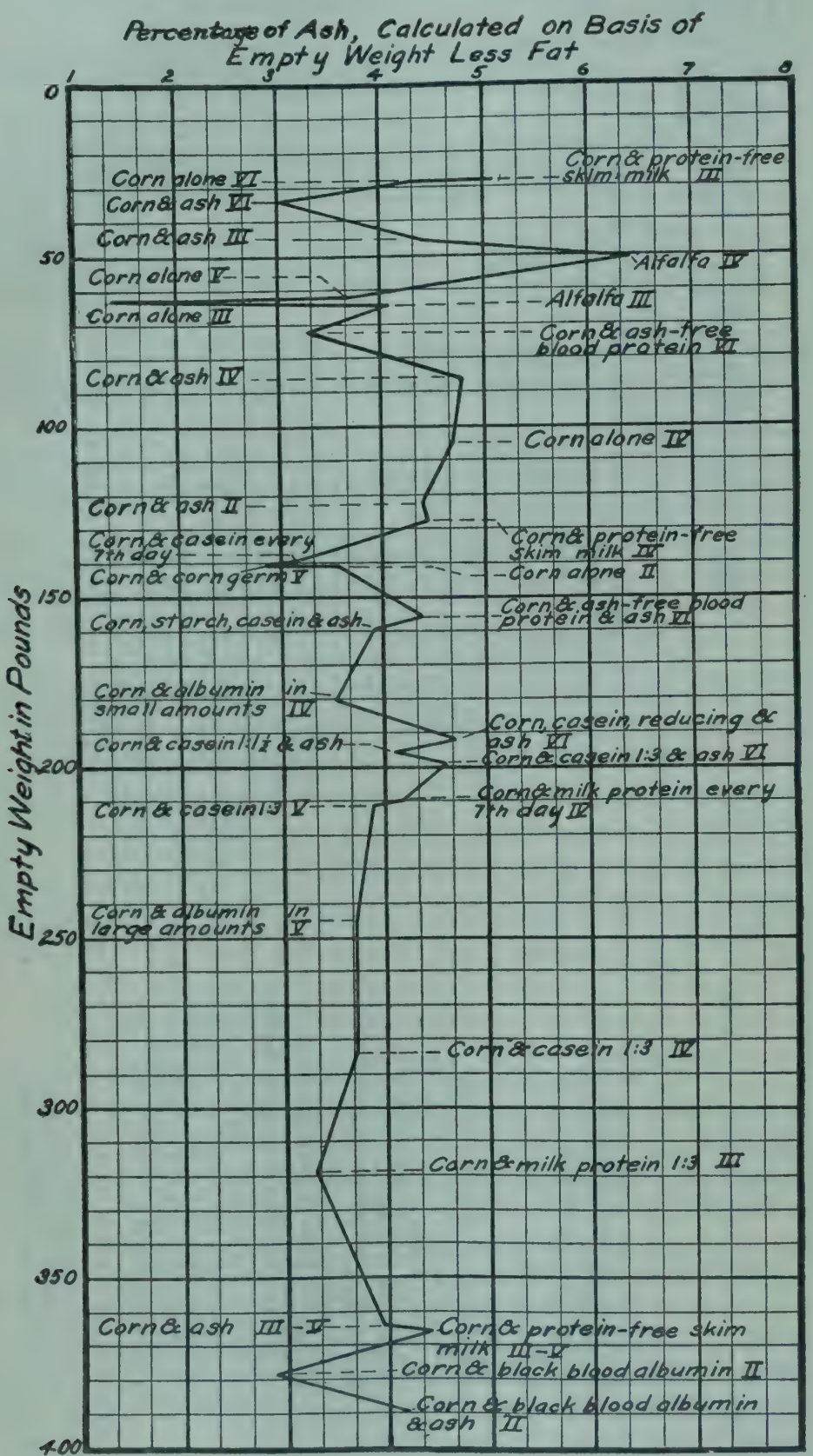

FIG 4.—Graph showing the relation between percentage of ash, calculated on basis of empty weight less fat, and empty weight.

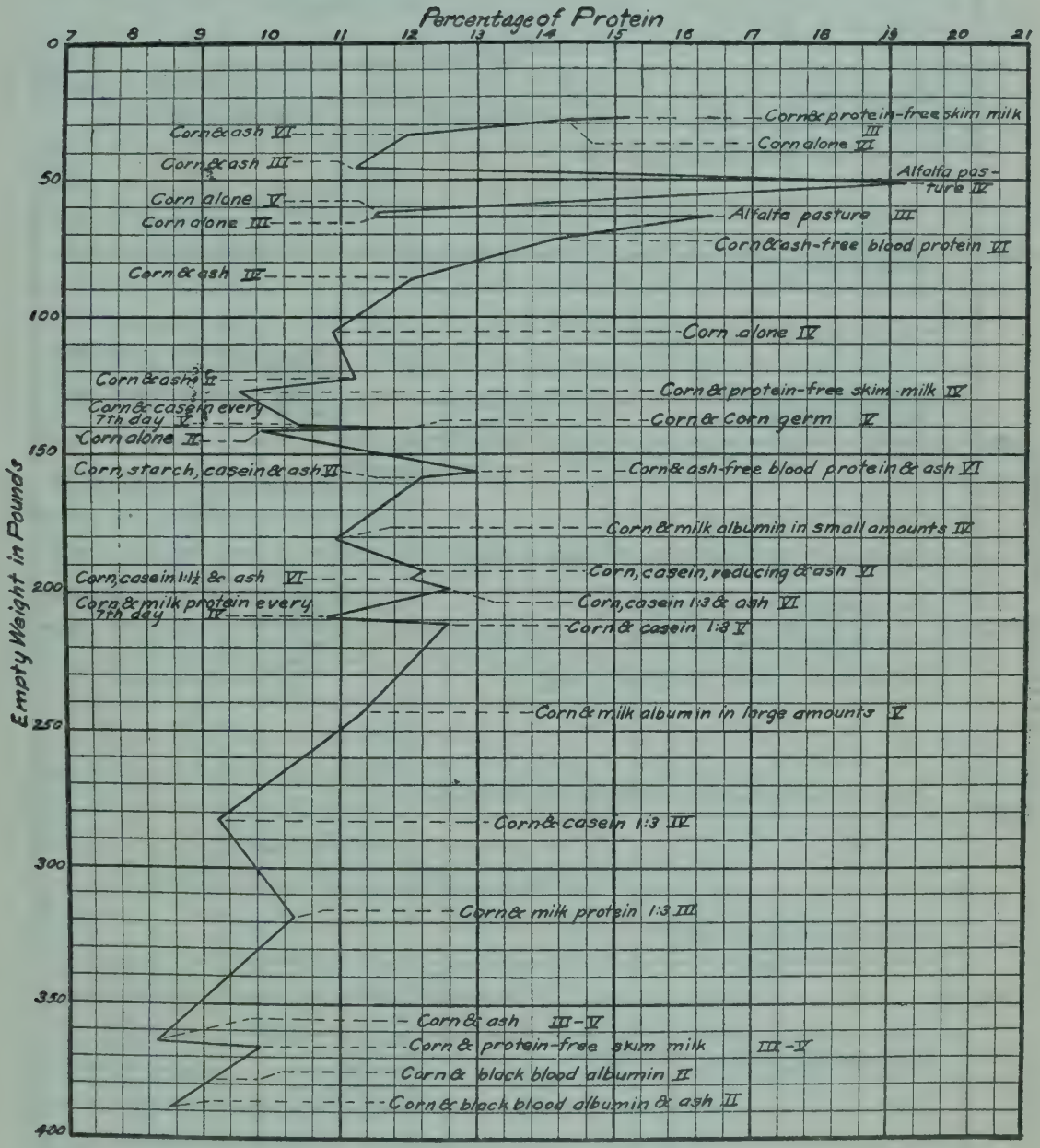
Percentage of Protein
0 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Empty Weight in Pounds
50
100
150
200
250
300
350
400
Corn & ash VI
Corn & ash III
Corn alone V
Corn alone III
Corn & ash III
Corn & ash II
Corn & casein every 7th day V
Corn alone II
Corn, starch, casein & ash VI
Corn, casein 1:1½ & ash VI
Corn & milk protein every 7th day IV
Corn & protein-free skim milk III
Corn alone VI
Alfalfa pasture IV
Alfalfa pasture III
Corn & ash-free blood protein VI
Corn alone IV
Corn & protein-free skim milk IV
Corn & Corn germ V
Corn & ash-free blood protein & ash VI
Corn & milk albumin in small amounts III
Corn, casein, reducing & ash VI
Corn, casein 1:3 & ash VI
Corn & casein 1:3 V
Corn & milk albumin in large amounts V
Corn & casein 1:3 IV
Corn & milk protein 1:3 III
Corn & ash III-V
Corn & protein-free skim milk III-V
Corn & black blood albumin II
Corn & black blood albumin & ash II

FIG. 5.—Graph showing the relation between percentage of protein and empty weight.

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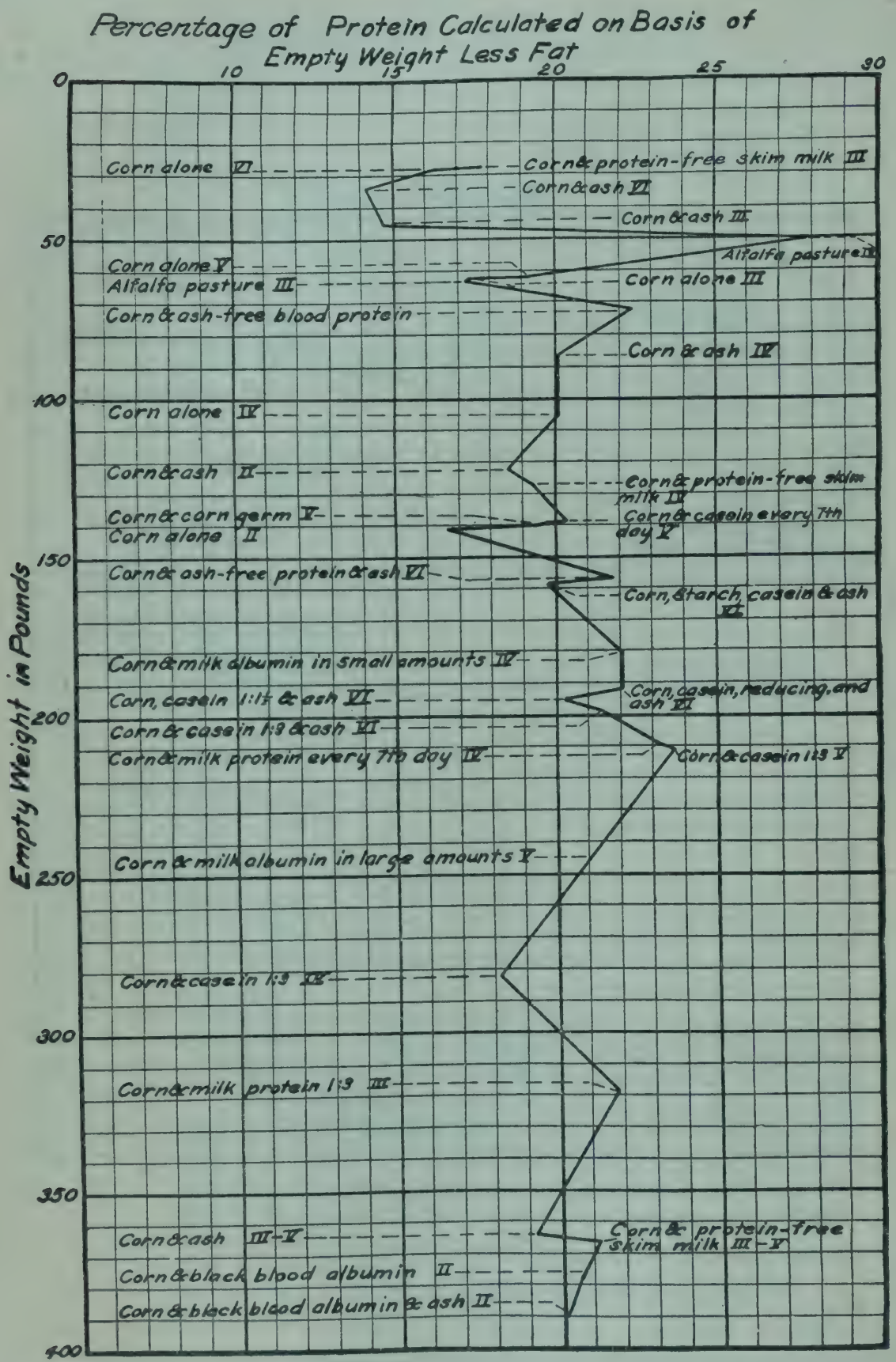
Percentage of Protein Calculated on Basis of Empty Weight Less Fat
0
10
15
20
25
30
50
100
150
200
250
300
350
400
Empty Weight in Pounds
Corn alone VI
Corn & protein-free skim milk III
Corn & ash VI
Corn & ash III
Alfalfa pasture II
Corn alone V
Alfalfa pasture III
Corn alone III
Corn & ash-free blood protein
Corn & ash IV
Corn alone IV
Corn & ash II
Corn & protein-free skim milk III
Corn & corn germ V
Corn & casein every 7th day V
Corn alone II
Corn & ash-free protein & ash VI
Corn, starch, casein & ash VI
Corn & milk albumin in small amounts IV
Corn, casein 1:1½ & ash VI
Corn, casein, reducing, and ash VI
Corn & casein 1:3 & ash VI
Corn & milk protein every 7th day IV
Corn & casein 1:3 V
Corn & milk albumin in large amounts V
Corn & casein 1:3 IV
Corn & milk protein 1:3 III
Corn & ash III-V
Corn & protein-free skim milk III-V
Corn & black blood albumin II
Corn & black blood albumin & ash II

FIG. 6.—Graph showing the relation between percentage of protein, calculated on basis of empty weight less fat, and empty weight.

4. In a pig fed a ration sufficient to produce a large and rapid growth, producing a body weight above 225 pounds, the fat percentage increases as the weight increases, and the moisture percentage decreases. The prolonged feeding of a deficient ration produces the same result—namely, a pig with a high percentage of fat and a low percentage of moisture, provided the pig attains large size.

5. The percentage of protein appears to decrease as the pigs increase in size. This is due to the large increase in percentage of fat. If the percentage of protein is calculated on the basis of body weight less fat, the percentage does not show any tendency to decrease as pigs increase in size.

6. The percentage of ash appears to decrease with the increasing body weight. This is due to the large proportionate increase in fat. If the ash percentage is calculated on body weight less fat, the larger pigs have as high an ash content in the tissues not fat as the lighter pigs.

7. The general character of the ration affects the composition of the body as follows: A large amount of protein or ash will not produce a pig with a high protein or ash content. Such a ration will produce a rapid growth, and the pig will have the same composition as a pig of the same size fed a ration restricted in protein or ash but whose growth took place over a longer period of time. The best illustrations of this fact are the two continuation hogs, one fed corn and ash and the other corn and protein-free skim milk, in comparison with the hogs in experiment II, in which one was fed corn and black blood albumen and the other the same ration plus ash.

8. A large amount of ash in the ration will not materially influence the percentage of ash in the body. The ash percentage in pigs fed rations sufficient for growth tends to remain fairly constant, if the percentage is calculated on the basis of body weight less fat.

9. There is a closer relation between a pig's size and its composition than between the character of the ration and the pig's composition. If growth has not been too much restricted, the composition of a slaughtered pig of a given weight may be used to calculate the pounds of chemical constituents in another pig not slaughtered. However, this must be done with judgment based upon knowledge regarding the history and condition of the pigs.

II. CHARACTER OF RATION IN ITS EFFECT ON THE DEVELOPMENT OF LUNGS, HEART, LIVER, AND KIDNEYS

That the character of the ration in respect to its nutritive properties has the same relative effect on the development of the internal organs as on the development of the body as a whole is borne out by evidence furnished by a number of investigators. Emmett (*2*) and associates fed three lots of pigs on low, medium, and high protein rations. The amounts

of digestible protein per day per 100 pounds live weight for the three lots were, respectively, 0.32 pound, 0.70 pound, and 0.94 pound.

> The weights of the kidneys of the pigs of the low protein lot were about 50 per cent lower than those of the pigs of the medium and high protein lots. The data for the other urinary organs and for the organs of the central nervous system did not show any lot differences that seemed significant. . . . The weights of the heart, liver, spleen, pancreas, gall bladder, and lungs of the pigs from the three lots varied more or less directly with the live weight of the animals.

Carroll and Emmett (*1*) report investigations on 21 lambs, divided into three lots, fed on (1) low, (2) medium, and (3) high protein planes.

> Slaughter tests showed that with the exception of the kidneys, the feed has little or no effect on the development of the brain, kidneys, liver, and heart. The kidneys seemed to increase directly with the protein feed. Individuality seems to be a very great factor, in fact, greater than the feed.

Forbes (*3*) notes that with pigs fed corn alone the liver, kidneys, lungs, and heart all compose an abnormally small proportion of the increase and the fat composes an abnormally large proportion of the increase. Sanborn (*8*, *9*, *10*) concludes from his investigations that the corn-fed hogs had a less abundant coat of hair, smaller spleen, liver, and kidneys, and smaller bones. Henry (*6*) found that the corn-fed hogs had an abnormally small amount of hair and a thin skin. The spleen, liver, and kidneys were unusually small, while the brain, heart, and lungs were not affected.

The slaughter tests made on the pigs fed the various rations, the results of which are recorded in Table VI, furnished abundant material for the study of the effect of these rations on the development of the internal organs—heart, lungs, liver, and kidneys. At the time of slaughter, these organs were weighed as soon as removed from the body on a scale accurate to 0.01 pound. The accuracy of the weights depends, of course, on the uniformity in separating these organs from their supporting tissues at relatively the same place. This involves considerable error, as is well known to all who are familiar with this class of work. Just how large this error is can not be determined. If a large number of tests were made on similar pigs, these variations would be eliminated in the average.

The figures as obtained are given in Table VI, and they are arranged in order of increasing weights. A study of these figures will show that, on the whole, these organs increase in weight as the body increases in weight, but not in the same proportion. The smaller pigs have relatively larger organs. This is due to the increase in fat in the larger pigs. The increase in fat takes place faster than the increase in any other constituent or parts of the body. But even allowing for this, the larger pigs do not uniformly have larger organs than smaller pigs. Thus, variations can not be correlated with the different rations fed and must be due to the individuality of the animals. From the results of the analysis of the

pig the pounds of protein were calculated. These weights of protein, arranged in the order of increasing weights, are found in Table VII. This same table gives also the ratio between the weight of protein and the weight of the internal organs.

TABLE VI.—*Relation of the weight of lungs, heart, liver, and kidneys to the empty weight*

Experiment No.	Ration.	Empty weight.	Lungs.	Heart.	Liver.	Kidneys.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
III.....	Corn and protein-free skim milk.	28.06	1.68	0.25	0.88	0.15
VI.....	Corn alone.....	28.60	1.04	.16	1.04	.12
VI.....	Corn and ash.....	34.37	.82	.18	.84	.10
III.....	.....do.....	45.62	.68	.16	.74	.13
IV.....	Alfalfa pasture.....	51.25	.82	.22	1.12	.24
V.....	Corn alone.....	62.71	.92	.24	1.54	.24
III.....	.....do.....	63.28	.53	.17	.56	.11
III.....	Alfalfa pasture.....	63.60	.98	.23	1.61	.30
VI.....	Corn and ash-free blood protein.	72.18	.94	.24	1.20	.28
IV.....	Corn and ash.....	86.40	1.59	.26	1.58	.17
IV.....	Corn alone.....	105.25	2.06	.36	1.56	.20
II.....	Corn and ash.....	122.80	1.36	.32	2.14	.24
IV.....	Corn and protein-free skim milk.	127.45	1.38	.36	2.36	.22
V.....	Corn and casein every seventh day.	139.84	1.08	.46	2.64	.30
V.....	Corn and corn germ.....	140.44	1.04	.42	2.78	.34
II.....	Corn alone.....	141.49	1.56	.50	2.06	.32
VI.....	Corn, ash-free blood protein, and ash.	156.86	.96	.32	2.60	.46
VI.....	Corn, starch, casein, and ash...	159.35	1.42	.40	2.56	.38
IV.....	Corn and albumin (small amounts).	180.99	1.35	.49	2.17	.29
VI.....	Corn, casein reducing, and ash..	192.20	1.22	.36	3.04	.46
VI.....	Corn, casein 1 to 1½, and ash...	195.66	1.34	.44	3.36	.50
VI.....	Corn, casein 1 to 3, and ash.....	199.71	1.65	.38	3.04	.69
IV.....	Corn and milk protein every seventh day.	209.95	1.44	.91	1.90	1.06
V.....	Corn and casein 1 to 3.....	211.22	1.52	.66	2.98	.58
V.....	Corn and albumin (large amounts).	245.15	1.62	.72	3.02	.44
IV.....	Corn and casein 1 to 3.....	282.58	2.82	.72	3.66	.50
III.....	Corn and milk protein 1 to 3...	318.93	2.89	.63	4.81	.95
III–V...	Corn and synthetic ash.....	364.52	1.90	1.00	5.40	.50
III–V..	Corn and protein-free skim milk.	366.42	2.08	.96	4.86	.58
II.....	Corn and black-blood albumen.	378.20	2.62	.88	4.34	.62
II.....	Corn, black-blood albumen, and ash.	389.26	2.55	.98	4.00	.85

A study of Table VII shows that:

1. In general the weights of these internal organs increase with the increase in weight of protein.

2. The variations are irregular and do not seem to have any specific relation to the character of the ration. The variation from the normal must be due to the individuality of the animal.

3. A ration which contains the nutrients for the normal development of the pig will develop the internal organs in the same ratio.

TABLE VII.—*Relation of the weight of the lungs, heart, liver, and kidneys to the weight of protein*

Experiment No.	Ration.	Protein.	Lungs to protein, 1 to	Heart to protein, 1 to	Liver to protein, 1 to	Kidneys to protein, 1 to
		Pounds.				
VI	Corn alone	4.09	3.93	25.56	3.93	34.08
VI	Corn and ash	4.15	5.06	23.07	4.94	41.50
III	Corn and protein-free skim milk.	4.27	2.54	17.08	4.95	28.48
III	Corn and ash	5.14	7.56	32.13	6.94	39.53
V	Corn alone	7.20	7.85	30.02	4.68	30.02
III	do	7.22	13.65	42.45	12.92	65.63
VI	Corn and ash-free blood protein.	10.19	10.80	42.49	8.49	10.19
IV	Corn and ash	10.35	6.51	39.85	6.55	60.86
III	Alfalfa pasture	10.44	10.65	45.41	6.48	34.77
IV	Corn alone	11.34	5.50	31.53	7.27	56.70
IV	Alfalfa pasture	11.64	14.20	52.96	10.39	48.53
IV	Corn and protein-free skim milk.	12.11	8.77	33.67	5.13	55.10
II	Corn and ash	12.67	9.32	39.66	5.92	52.83
II	Corn alone	12.81	8.21	25.62	6.22	40.10
V	Corn and casein every seventh day.	14.61	13.53	31.70	5.53	48.70
V	Corn and corn germ	16.90	16.25	40.22	6.08	49.69
VI	Corn, starch, casein, and ash	19.45	13.70	48.63	7.60	51.15
IV	Corn and milk albumin (small amounts).	19.50	14.44	39.78	8.99	67.28
VI	Corn, ash-free blood protein, and ash.	20.43	21.25	63.95	7.86	44.33
IV	Corn and milk protein every seventh day.	22.59	14.30	24.85	11.89	21.31
VI	Corn, casein 1 to 1½, and ash	23.54	17.57	53.44	7.01	47.08
VI	Corn, casein reducing, and ash	23.64	19.38	65.72	7.78	51.30
VI	Corn, casein 1 to 3, and ash	25.33	15.35	66.62	8.33	36.73
IV	Corn and casein 1 to 3	26.63	6.97	37.02	7.28	53.26
V	do	26.73	17.59	40.63	8.97	45.98
V	Corn and albumin (large amounts).	27.78	17.15	38.61	9.20	63.06
III–V	Corn and synthetic ash	30.34	15.97	30.34	5.61	60.68
II	Corn, black-blood albumen, and ash.	31.89	12.51	32.53	7.97	37.63
III	Corn and milk protein 1 to 3	33.08	11.45	52.60	6.88	34.82
II	Corn and black-blood albumen	33.59	12.82	38.29	7.74	33.59
III–V	Corn and protein-free skim milk.	36.16	17.39	37.61	7.44	62.20

LUNG WEIGHTS IN RELATION TO EMPTY WEIGHT AND WEIGHT OF PROTEIN

The figures for the weight of lungs in relation to empty weight are plotted in figure 7, and those in relation to weight of protein in figure 8. The empty weights and the weights of protein are used as the abscissas and the weights of the organs as ordinates. The weights of protein were approximately one-tenth of the empty weight, and to make the curves more comparable the protein figures were multiplied by 10. As pigs increase in size or in amount of protein, the lungs increase irrespective of the nature of the ration.

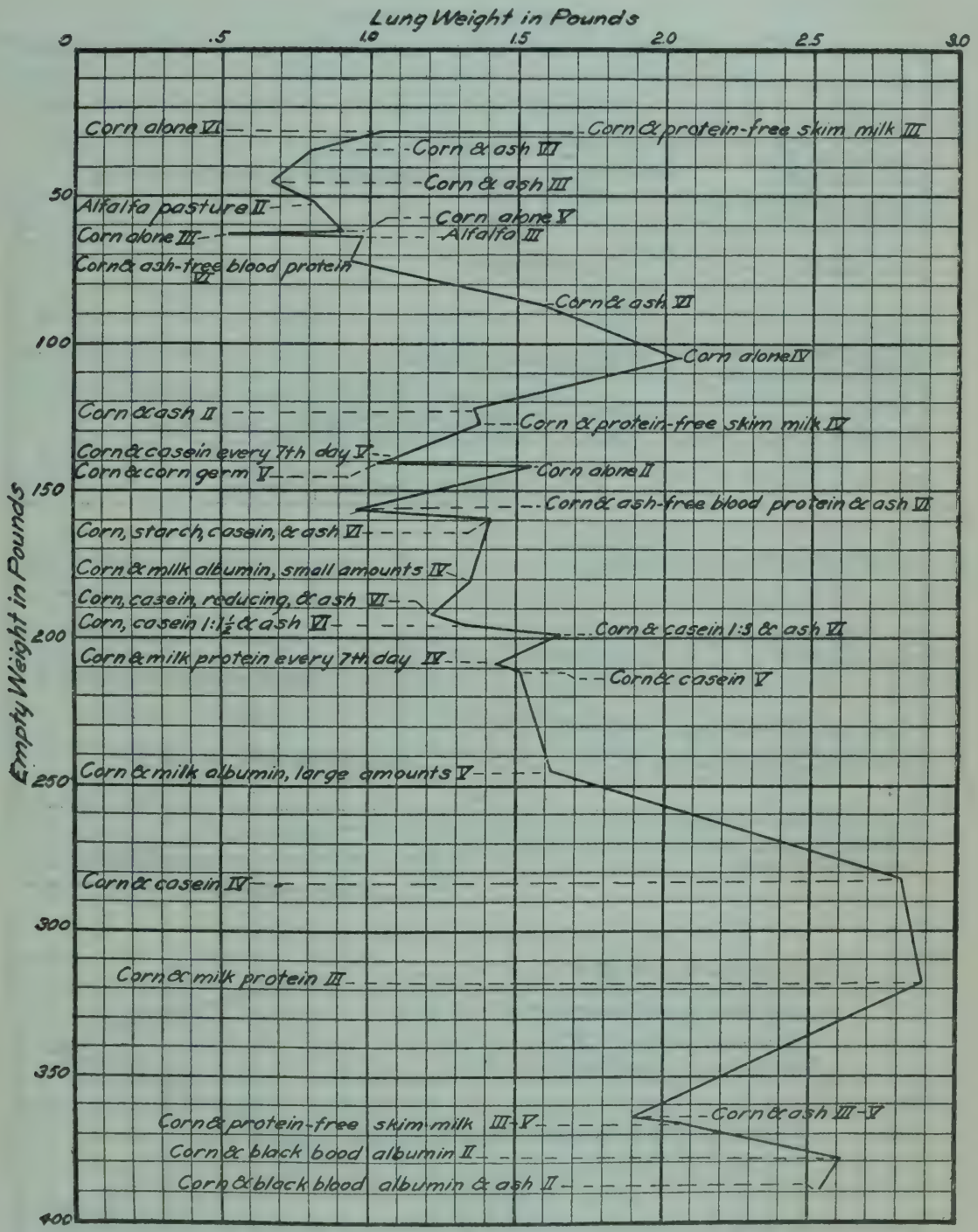

FIG. 7.—Graph showing the relation between weight of lungs and empty weight.

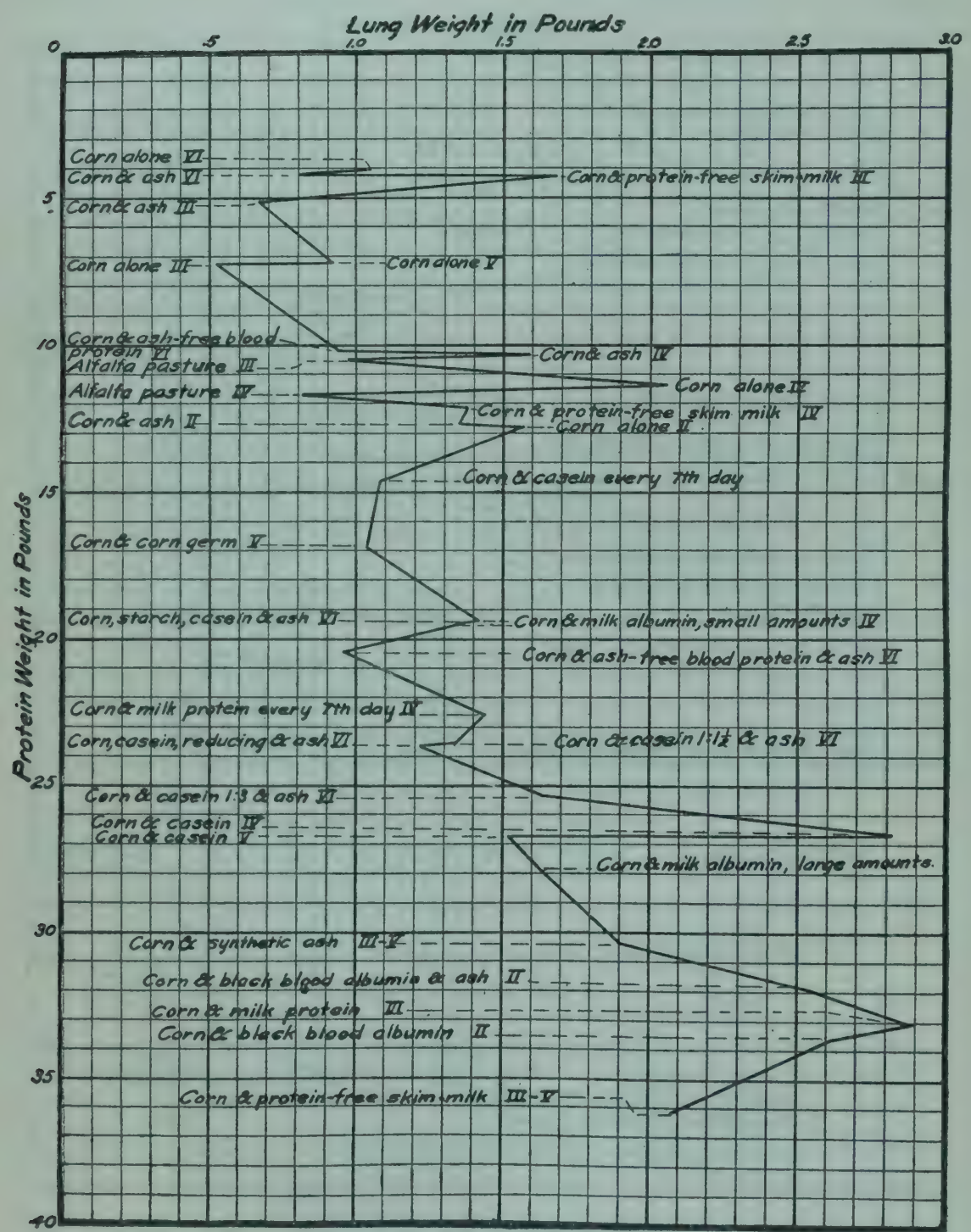

FIG. 8.—Graph showing the relation between weight of lungs and weight of body protein.

HEART WEIGHTS IN RELATION TO EMPTY WEIGHT AND TO WEIGHT OF PROTEIN

The figures for the weight of the heart in relation to empty weight are plotted in figure 9, and in relation to protein weights in figure 10. The figures for empty weight and protein weight are used as abscissas, and the weights of heart, expressed in tenths of a pound, are used as ordinates. The weight of the heart increases as the size of the body or as the amount of protein increases even more regularly than the weight of lungs.

WEIGHT OF THE LIVER IN RELATION TO EMPTY WEIGHT AND WEIGHT OF PROTEIN

The curve for the weights of the liver in relation to empty weight is plotted in figure 11, and in relation to protein weights in figure 12. The weights of the liver seem to vary in proportion to the empty weight or the weight of the protein more than any of the other organs studied.

KIDNEY WEIGHT IN RELATION TO EMPTY WEIGHT AND WEIGHT OF PROTEIN

The relation between the weight of the kidneys and the empty weight, and also between the kidney weight and the weight of protein, is graphically presented in figures 13 and 14. The kidneys become larger as the pigs increase in size, and this increase in size is very irregular. There seems to be no correspondence between the character of ration and the size of kidney. The largest kidneys weighed 1.06 pounds and were found in the pig fed corn and milk protein every seventh day. The kidneys in the pig in the same experiment fed seven times this amount of protein weighed 0.95 pound.

CONCLUSIONS

1. The specific character of the ration has, in general, the same effect on the development of the internal organs as on the development of the body as a whole.

2. A ration that will produce a large development of the body as a whole will also produce large development of the internal organs.

3. The relative smallness of the internal organs in some pigs of restricted growth is due to the overdevelopment of fat.

4. In general, the development of internal organs is also in proportion to the increase of protein. A ration that will produce a large amount of protein will produce large internal organs.

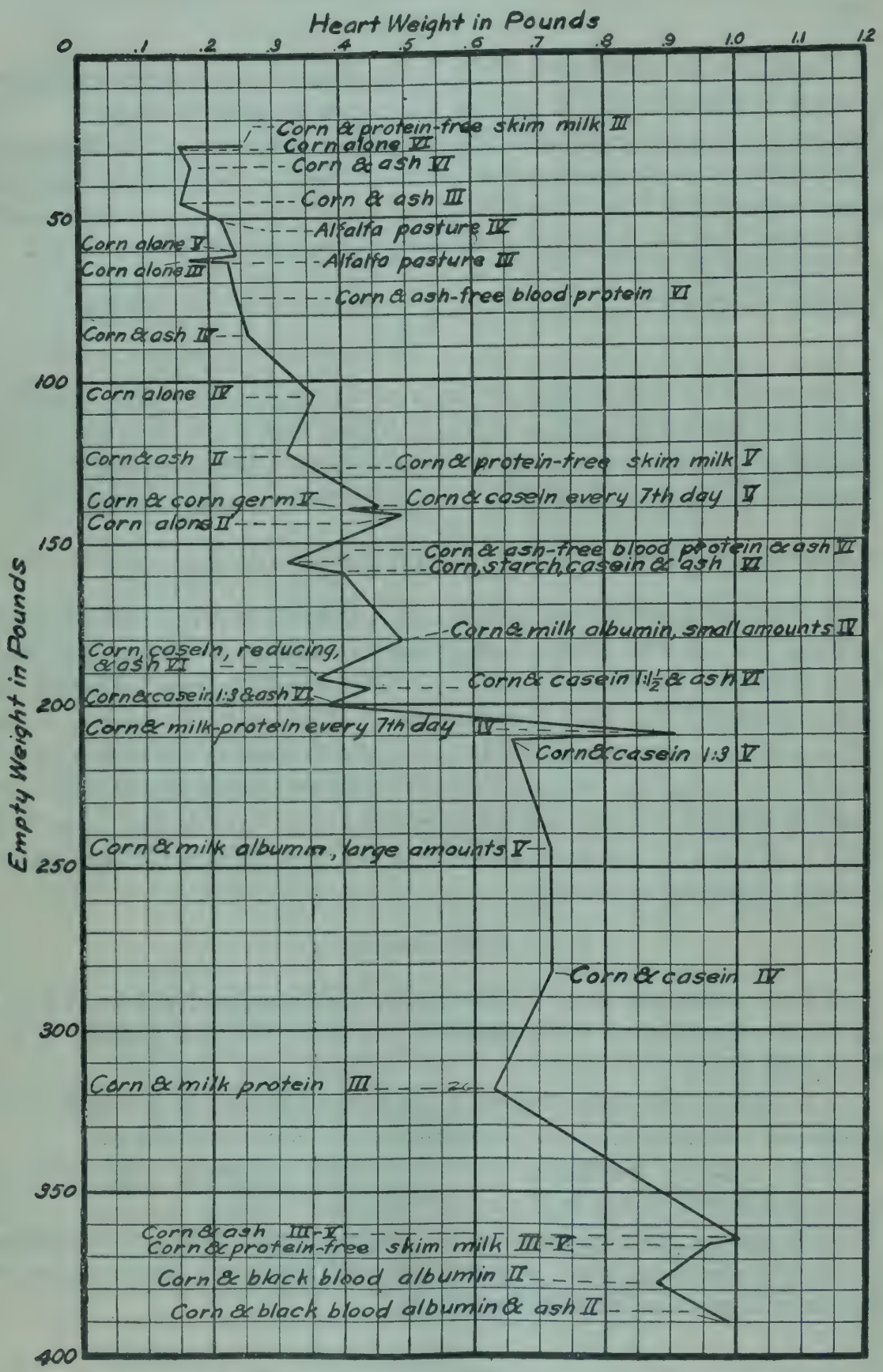

FIG. 9.—Graph showing the relation between weight of heart and empty weight.

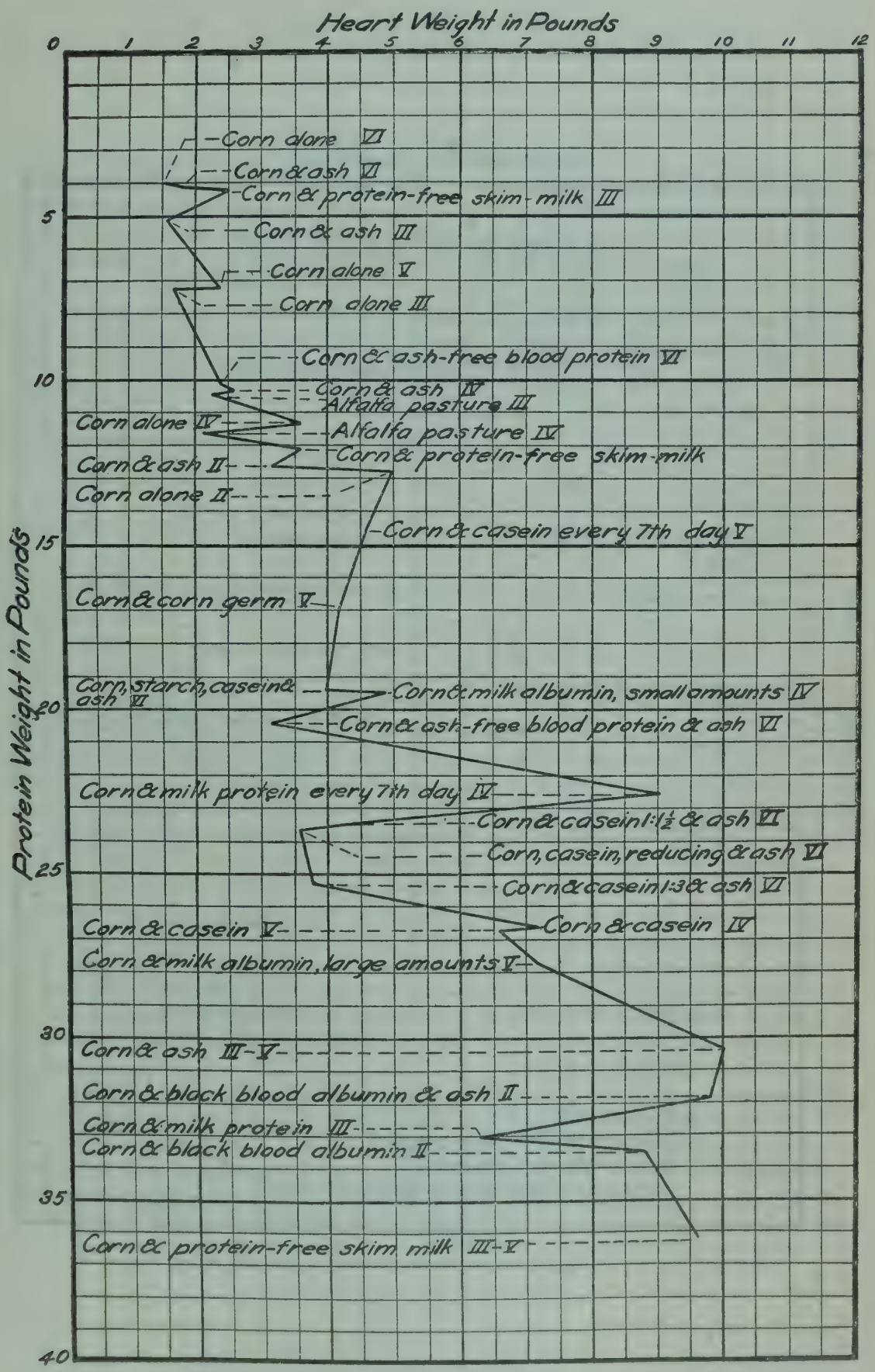
Heart Weight in Pounds
0 1 2 3 4 5 6 7 8 9 10 11 12
Protein Weight in Pounds
5 10 15 20 25 30 35 40
Corn alone VI
Corn & ash VI
Corn & protein-free skim-milk III
Corn & ash III
Corn alone V
Corn alone III
Corn & ash-free blood protein VI
Corn & ash IV
Alfalfa pasture III
Corn alone IV
Alfalfa pasture IV
Corn & protein-free skim-milk
Corn & ash II
Corn alone II
Corn & casein every 7th day V
Corn & corn germ V
Corn, starch, casein & ash VI
Corn & milk albumin, small amounts IV
Corn & ash-free blood protein & ash VI
Corn & milk protein every 7th day IV
Corn & casein 1:1½ & ash VI
Corn, casein, reducing & ash VI
Corn & casein 1:3 & ash VI
Corn & casein V
Corn & casein IV
Corn & milk albumin, large amounts V
Corn & ash III-V
Corn & black blood albumin & ash II
Corn & milk protein III
Corn & black blood albumin II
Corn & protein-free skim milk III-V

FIG. 10.—Graph showing the relation between weight of heart and weight of body protein.

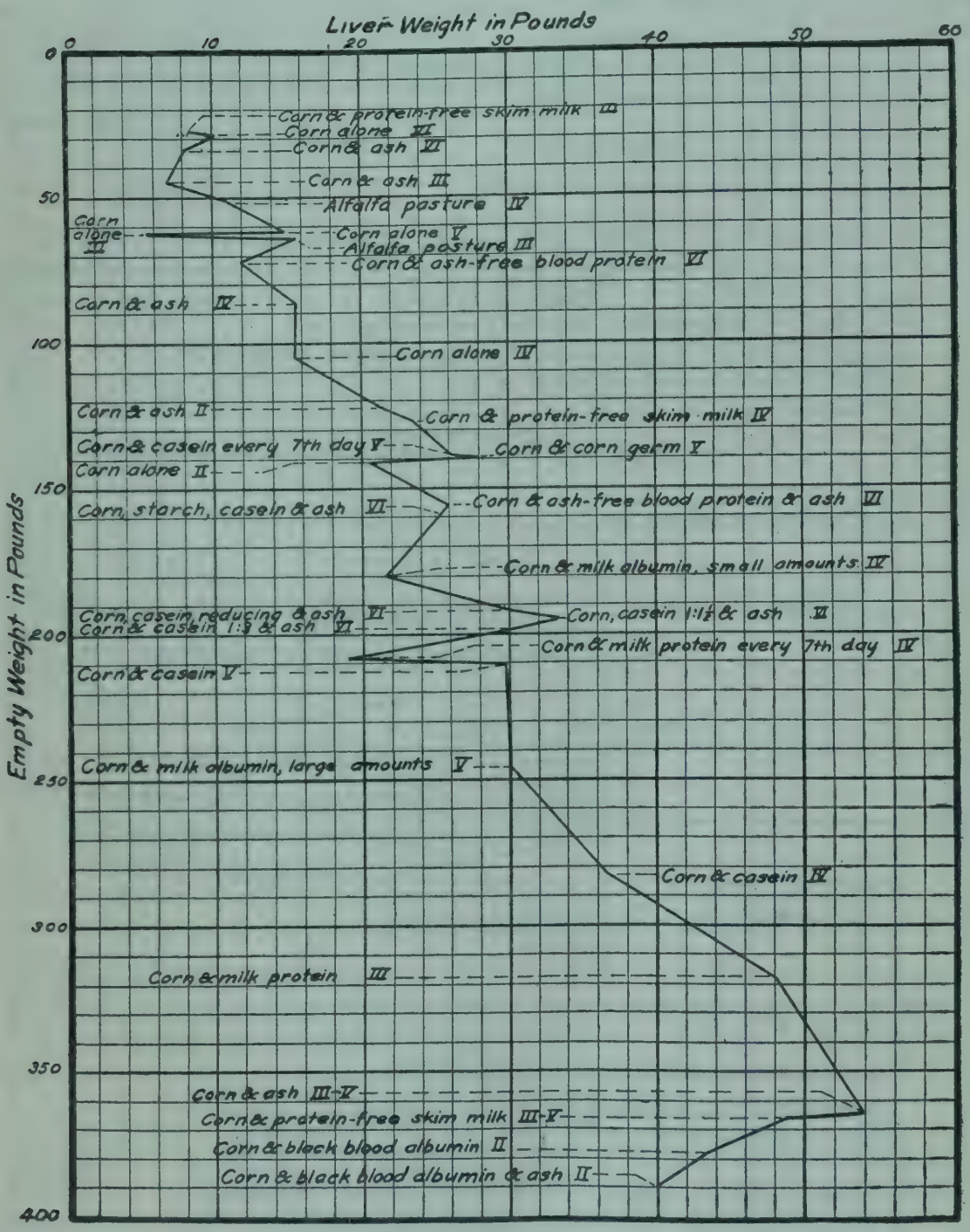

FIG. 11.—Graph showing the relation between weight of liver and empty weight.

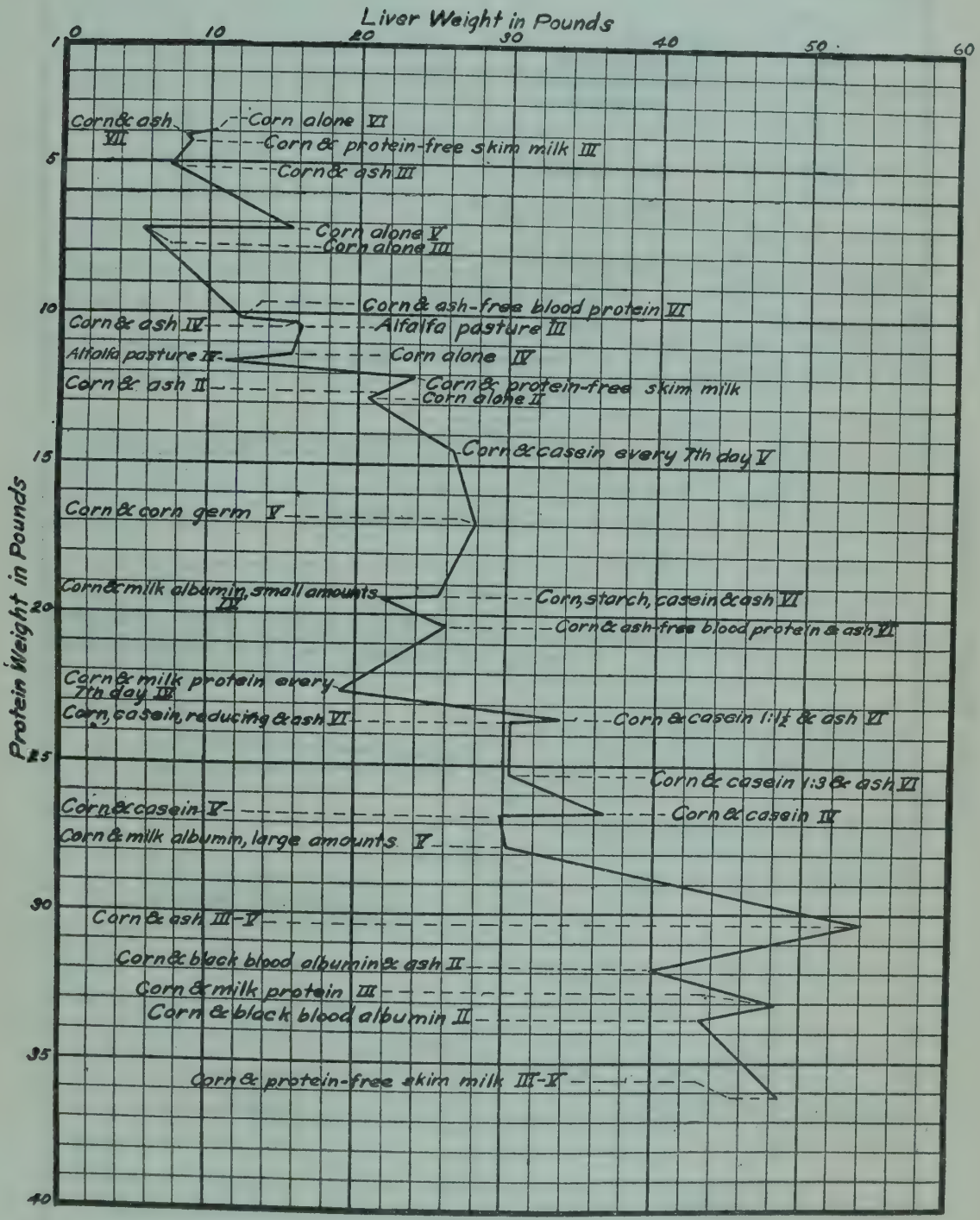

FIG. 12.—Graph showing the relation between weight of liver and weight of body protein.

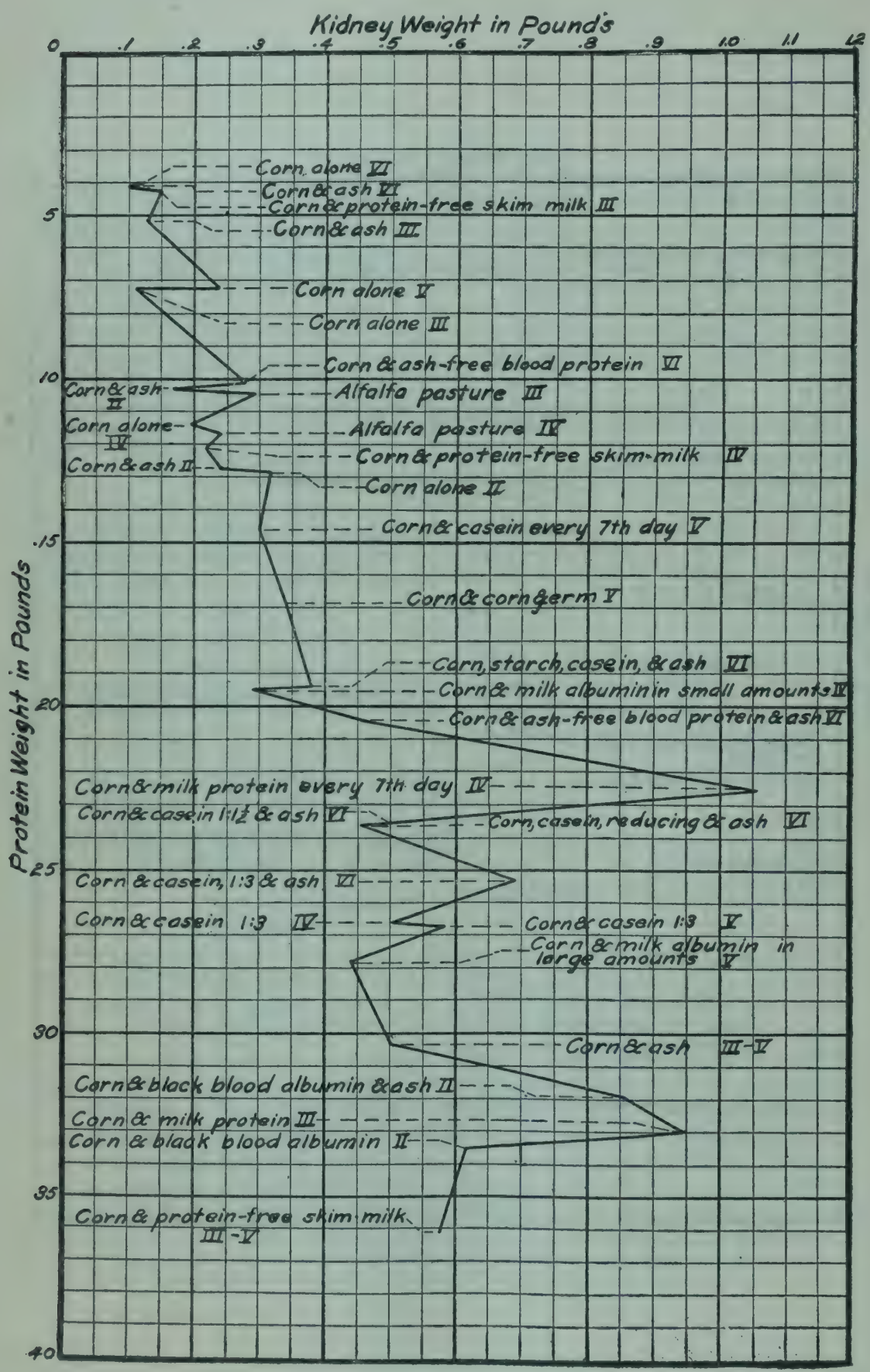

FIG. 13.—Graph showing the relation between weight of kidney and empty weight.

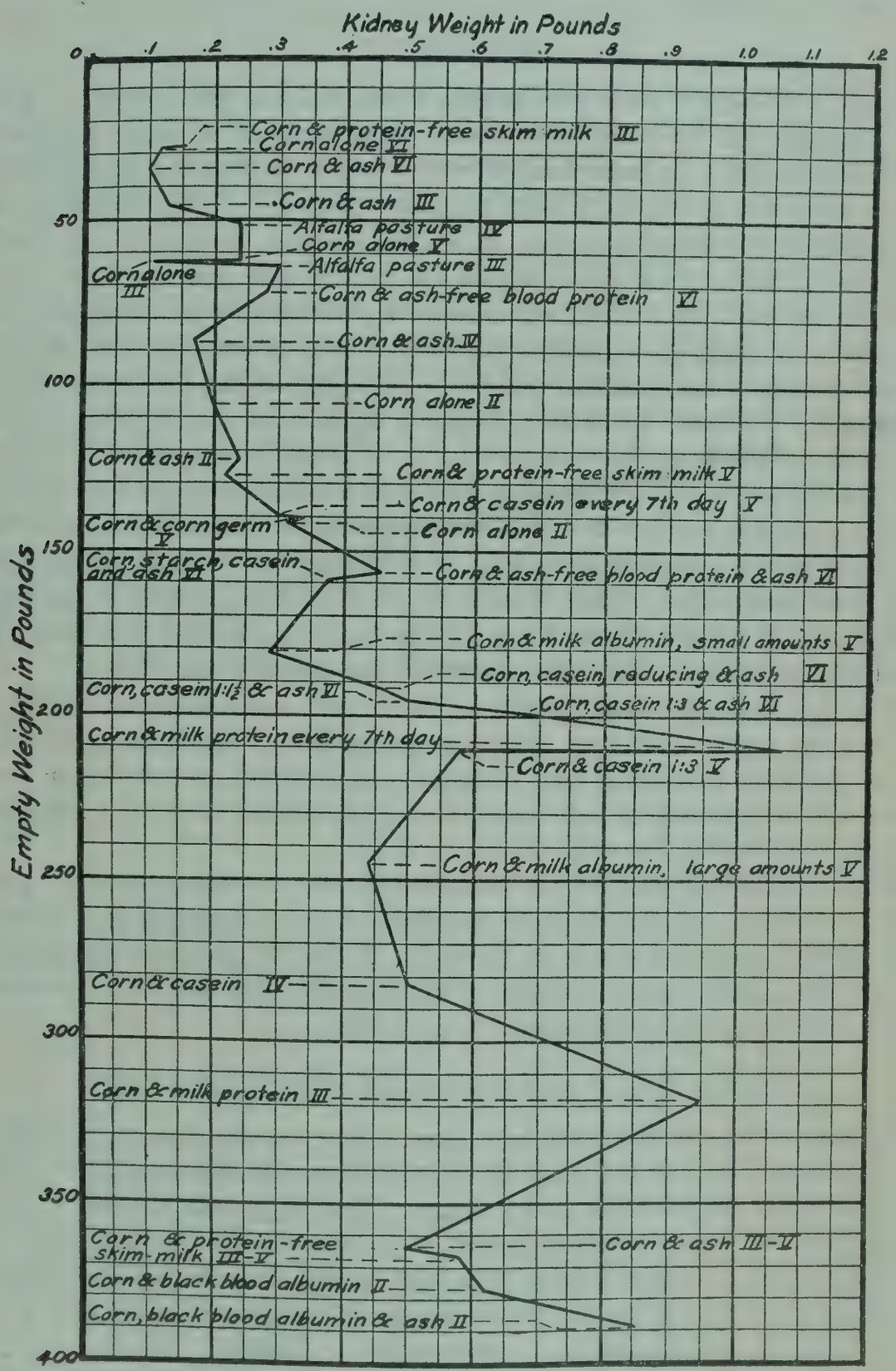

FIG. 14.—Graph showing the relation between weight of kidney and weight of body protein.

III. RELATION BETWEEN THE AMOUNT OF CHEMICAL CONSTITUENTS CONSUMED DURING THE FEEDING TRIALS AND THE AMOUNT STORED

THE PROBLEM

A pig's value for meat production depends upon the amount of material stored in the body in relation to the amount of feed consumed. The problem may be stated as follows: A pig consumes during a feeding trial a certain amount of ash, fat, and protein. The problem is to calculate the relation between these two sets of quantities and state the results in common units, which are in this case pounds. The composition of each pig at the beginning of a given trial was assumed to be the same as that of a control pig slaughtered when the trial started. The amount of ash, protein, and fat in each pig at the beginning of the trial was calculated by multiplying the figures for the composition of the control pig into the weights of the different pigs used in the trial. At the end of each trial the most representative pig in a lot was slaughtered. On the basis of the chemical composition and the weight of the emptied body, the pounds of ash, protein, and fat in each slaughtered pig were calculated. The differences between the pounds of protein, ash, and fat present in each pig at the beginning of the trial and the amounts at the end of the trial represent the amount stored.

The amount and composition of each feed consumed by the pigs during the entire trial were carefully determined. From the figures on total amounts consumed and chemical composition, a calculation was made of the pounds of ash, protein, carbohydrates, and fat consumed by each pig during the trial.

The detailed figures on the amount of the different chemical constituents from the several feeds consumed by the different pigs during the feeding trials are given in Table I. Table III gives the figures for composition and empty weights of the control pigs and the representative pig slaughtered at the end of each trial.

PROTEIN STORED IN RELATION TO PROTEIN CONSUMED

Protein stored in the body can come only from the digestible protein in the feed. Not all the protein digested from the feed can be stored; a part must be used for maintenance. The digestible protein in the feed must be equal to the amount of protein needed for maintenance and the amount that is stored, plus such amounts as are in forms which can not be used for these two purposes. The amount of protein that can be used for storage and maintenance is limited. If more is fed than is needed for these two purposes it may be used for the production of heat and energy, or fat. A protein is made up of a number of amino acids. These acids must be present in the feed in the proportion required by the needs of the body. The amount of protein that can be

stored in the body and the amount that can be used for maintenance are measured by the minimal amount of any amino acid absolutely required. A protein that has a small proportion of any amino acid absolutely needed is less efficient than one in which the different amino acids are so balanced that they meet the requirements of the body. A protein that has a small amount of any amino acid absolutely needed is efficiently supplemented by another protein which has a relatively large proportion of this particular amino acid.

FIVE PIGS THAT WERE STARVED

The experiments reported herewith extended over a period of five years. The same feed gave different results in different years. The individuality of pigs played a part. There were five pigs in these different trials which either lost weight or made such small gains that they were evidently starving. Two pigs, one fed corn and ash and the other fed corn and protein-free skim milk in experiment III, lost protein from the body. Three pigs, one fed corn and ash and one fed corn alone (experiment VI), and also one pig fed corn alone (experiment III), stored very small amounts of protein in proportion to the amount fed. These last three stored only 1 pound of protein for 24 pounds consumed. This is an abnormally wide ratio. These five pigs will be eliminated in the further discussion.

PROTEIN STORED FROM CORN FED ALONE OR WITH ASH

Zein is the protein in corn which occurs in larger amounts than any other. This protein is deficient in that it lacks the two amino acids, lysin and tryptophane, both necessary for growth and development.

TABLE VIII.—*Relation of protein stored to protein consumed*

Experiment No.	Ration.	Protein stored.	Protein consumed.		Ratio.
			From corn.	From supplement.	
		Pounds.	*Pounds.*	*Pounds.*	
V	Corn alone	3.76	27.25	.....	1:7.25
IV	Corn and ash	4.58	31.72	.....	1:6.92
IV	Corn alone	4.86	41.87	.....	1:8.62
IV	Corn and protein-free skim milk	5.36	37.17	3.24	1:7.54
II	Corn and ash	5.70	36.07	.....	1:6.32
II	Corn alone	6.30	42.58	.....	1:6.77
III–V	Corn and ash (continuation hog)	24.63	203.73	.....	1:8.30
III–V	Corn and protein-free skim milk (continuation hog)	29.75	190.75	16.03	1:6.95

There were three pigs fed on corn alone, three fed corn supplemented with ash, and two fed corn supplemented with protein-free skim milk. This substance contained a small amount of nitrogenouscompo unds

and most of the ash from the milk. The quantity obtained from 3 pounds of milk was fed for each pound of corn. These two pigs were continued on these rations until they were nearly 3 years old. Figures on the feed and growth of the eight pigs, showing the ratio of the protein stored to the protein consumed, are given in Table VIII.

The average ratio of the protein stored to the protein consumed for all these pigs is 1 to 7.5. As corn was practically the sole source of protein for all these pigs it means that when they are fed corn alone pigs will store 1 pound of protein for about 7.5 pounds consumed. This is a wide ratio. It is not so much that the protein in corn occurs in too small a proportion but that a large part of this protein is deficient in lysin and tryptophane. These occur in too small proportions in relation to the amount of other substances which can be used only for the production of fat.

The fact that the two pigs fed until nearly 3 years old on a deficient ration stored protein in the same ratio as the younger pigs should be noted. The fact that these pigs did not grow for over a year at the beginning of the trial means that there was a period in the life of these two pigs when the storage of protein in relation to the amount consumed was much less than the average ratio for the whole life period; and accordingly there must have been a time when the storage of protein took place in a much narrower ratio. The development of these two pigs will be studied in a subsequent section of this paper.

PROTEIN STORED FROM CORN SUPPLEMENTED WITH A SMALL AMOUNT OF PROTEIN FROM MILK

The two proteins in milk, casein and albumin, contain the two amino acids, lysin and tryptophane, both missing in zein. As milk is the natural food for young mammals, it is expected that the proteins in milk should contain the materials needed for growth. How much protein is needed from milk in order to supplement the protein in corn? Table IX gives the figures on the feed and growth of three pigs fed small amounts of supplementary milk protein and shows the ratios of protein stored to protein consumed.

TABLE IX.—*Relation of protein stored to protein consumed*

Experiment No.	Ration.	Protein stored.	Protein consumed.		Ratio.
			From corn.	From milk.	
		Pounds.	*Pounds.*	*Pounds.*	
IV.....	Corn and milk albumin.....	14. 14	59. 00	6. 71	1:4. 65
IV.....	Corn and milk protein every seventh day.....	15. 71	66. 60	8. 00	1:4. 75
V.....	Corn and casein every seventh day...	10. 26	55. 16	5. 61	1:5. 93

The milk albumin obtained from 3 pounds of milk was fed for each pound of corn. The seventh day means that corn was fed alone for six days and on the seventh day the total protein from 3 pounds of milk or the casein equivalent was fed for each pound of corn. These small amounts of supplementary protein, from one-eighth to one-tenth of the total protein furnished, resulted in a ratio of protein storage of 1 to 5.11, as compared with 1 to 7.5 when corn was fed alone.

PROTEIN STORED WHEN CORN WAS SUPPLEMENTED WITH A MEDIUM AMOUNT OF PROTEIN FROM MILK

There were two pigs fed proteins from milk in medium amounts. One was fed the casein from 1.5 pounds of milk for each pound of corn. This ration is designated corn and casein 1 to 1.5. The other pig was fed casein in such amounts that the nutritive ratio which at first was 1 to 3 was gradually widened until it was 1 to 8. The amount of protein from milk supplied to the pig fed corn and casein 1 to 1.5, was nearly two-sevenths of the total, and the other pig received nearly one-third of his protein from milk. These two pigs stored protein in a ratio half as wide as those pigs fed corn alone. This proportion of protein from milk was more efficient than greater or smaller amounts. Figures on the feed and growth of these two pigs showing the ratio of the protein stored to the protein consumed are shown in Table X.

TABLE X.—*Relation of protein stored to protein consumed*

Experiment No.	Ration.	Protein stored.	Protein consumed.		Ratio.
			From corn.	From milk.	
		Pounds.	*Pounds.*	*Pounds.*	
VI.....	Corn and casein, 1: 1.5.....	21. 47	51. 03	20. 81	1:3. 34
VI.....	Corn and casein decreasing.....	21. 45	53. 07	25. 84	1:3. 70

PROTEIN STORED WHEN CORN WAS SUPPLEMENTED WITH A LARGE AMOUNT OF PROTEIN FROM MILK

There were five pigs fed protein from milk in large amounts. The term milk protein here means both the casein and the albumin in the proportion in which they occur in milk. The milk protein or the casein from 3 pounds of milk was fed for each pound of corn. The milk albumin was fed in such amounts as to give practically the same amount of protein as the casein from 3 pounds of milk for each pound of corn. In experiment VI, the casein was fed so as to make a nutritive ratio of 1 to 3.3 The figures giving the ratio of protein stored to protein consumed for these five pigs are given in Table XI.

TABLE XI.—*Relation of protein stored to protein consumed*

Experiment No.	Ration.	Protein stored.	Protein consumed.		Ratio.
			From corn.	From milk.	
		Pounds.	*Pounds.*	*Pounds.*	
III.....	Corn and milk protein.....	27.33	90.54	89.39	1:6.58
IV.....	Corn and casein.....	20.18	84.65	66.65	1:7.49
V.....	.....do.....	21.84	65.72	50.72	1:5.33
V.....	Corn and milk albumin.....	24.30	77.04	46.32	1:5.08
VI.....	Corn and casein.....	22.90	47.35	80.70	1:5.59

The protein from milk varied from a little less than one-half to almost two-thirds of the total protein consumed. The amount of protein stored was from about one-half to about one-fourth of the protein supplied by the milk. This is in sharp contrast to the results where minimum or medium amounts of milk protein were fed. When milk protein was fed in minimum amounts the pounds of protein stored were about twice the pounds of protein supplied in the milk. When milk protein was fed in medium amounts the pounds of protein stored were nearly the same as the pounds of milk protein supplied. When milk protein was fed in large amounts, the amount of protein stored was from less than one-third to about one-half what was supplied in the milk. That is, from 3 pounds of milk protein supplied, in addition to what the corn furnished, less than 1 pound was stored. The average ratio of protein storage when large amounts were fed was 1 to 6, as compared with 1 to 3.5 when medium amounts of milk protein were fed or 1 to 7.5 when corn was fed alone.

PROTEIN STORED WHEN CORN WAS SUPPLEMENTED WITH PROTEIN IN LARGE AMOUNTS FROM VARIOUS SOURCES

The proteins from other sources than milk were black blood albumen, corn germ, and ash-free blood protein (p. 280–281). These rations were made up as follows: Black blood albumen constituted a little over 11 per cent of the ration having a nutritive ratio of 1 to 5.10; corn germ was about one-third of the ration having a nutritive ratio of 1 to 8; ash-free blood protein was fed in such amounts as to make a nutritive ration of 1 to 3; starch and casein were added in such amounts as to make a nutritive ratio the same as for corn, or 1 to 8.76. Figures on the feed and growth of six pigs fed on corn supplemented with protein from these sources as indicated, showing the ratio of protein stored to protein consumed, are as given in Table XII.

The wide nutritive ratios with corn and corn germ, or corn, starch, casein, and ash, resulted in the most economical storage of protein. This is in harmony with results presented in the preceding paragraphs.

TABLE XII.—*Relation of protein stored to protein consumed*

Experiment No.	Ration.	Protein stored.	Protein consumed.		Ratio.
			From corn.	From supplement.	
		Pounds.	*Pounds.*	*Pounds.*	
II.....	Corn and black blood albumen.....	26.28	105.97	129.21	1 : 8.95
II.....	Corn and black blood albumen and ash	24.27	118.20	142.25	1 : 10.73
V.....	Corn and corn germ.....	13.36	31.07	21.47	1 : 3.93
VI.....	Corn and ash-free blood protein.....	7.15	22.27	23.61	1 : 6.40
VI.....	Corn, ash-free blood protein, and ash.	18.24	39.29	41.66	1 : 4.43
VI.....	Corn, starch, casein, and ash.....	16.85	24.84	22.61	1 : 2.85

When corn was fed alone, the protein was stored in a ratio of 1 to 7.5; with corn germ this ratio was nearly halved, showing the superiority of the protein in the germ over the proteins in the kernel as a whole. The ration corn, starch, casein, and ash resulted in a larger proportion of protein storage in relation to the amount fed than any other ration in this entire series of experiments. It was a wide ratio, but nearly half of the protein came from milk.

The pigs fed the black blood albumen and the ash-free blood protein were fed protein in too large amounts, hence the wide ratio.

GRAPHIC PRESENTATION OF THE RELATION BETWEEN THE AMOUNT OF PROTEIN CONSUMED AND THE AMOUNT OF PROTEIN STORED

The quantitative relations between the amount of protein stored and the amounts of protein consumed are given in figure 15. The bars are arranged according to the increase in protein storage. These bars, representing the amount of protein stored, present a gradually rising curve. This is in contrast to the very irregular heights of the bars representing the amounts of protein consumed. This simply means that the power of the pig to store protein is limited. If protein is fed in small amounts and the protein is of the right kind, as milk protein fed in small amounts, then the ratio between the amount of protein consumed and the amount of protein stored will be narrow. When protein is fed in large amounts, the amount stored will not be increased in proportion no matter what the character of the protein, but it will be stored in a wide ratio.[1] The excess of protein fed will be used for the production of fat or energy. When supplementary proteins were fed in too small amounts, the ratio of protein storage was wider than when these amounts were approximately one-third of the total protein.

[1] The idea here presented should not be confused with rate of growth.

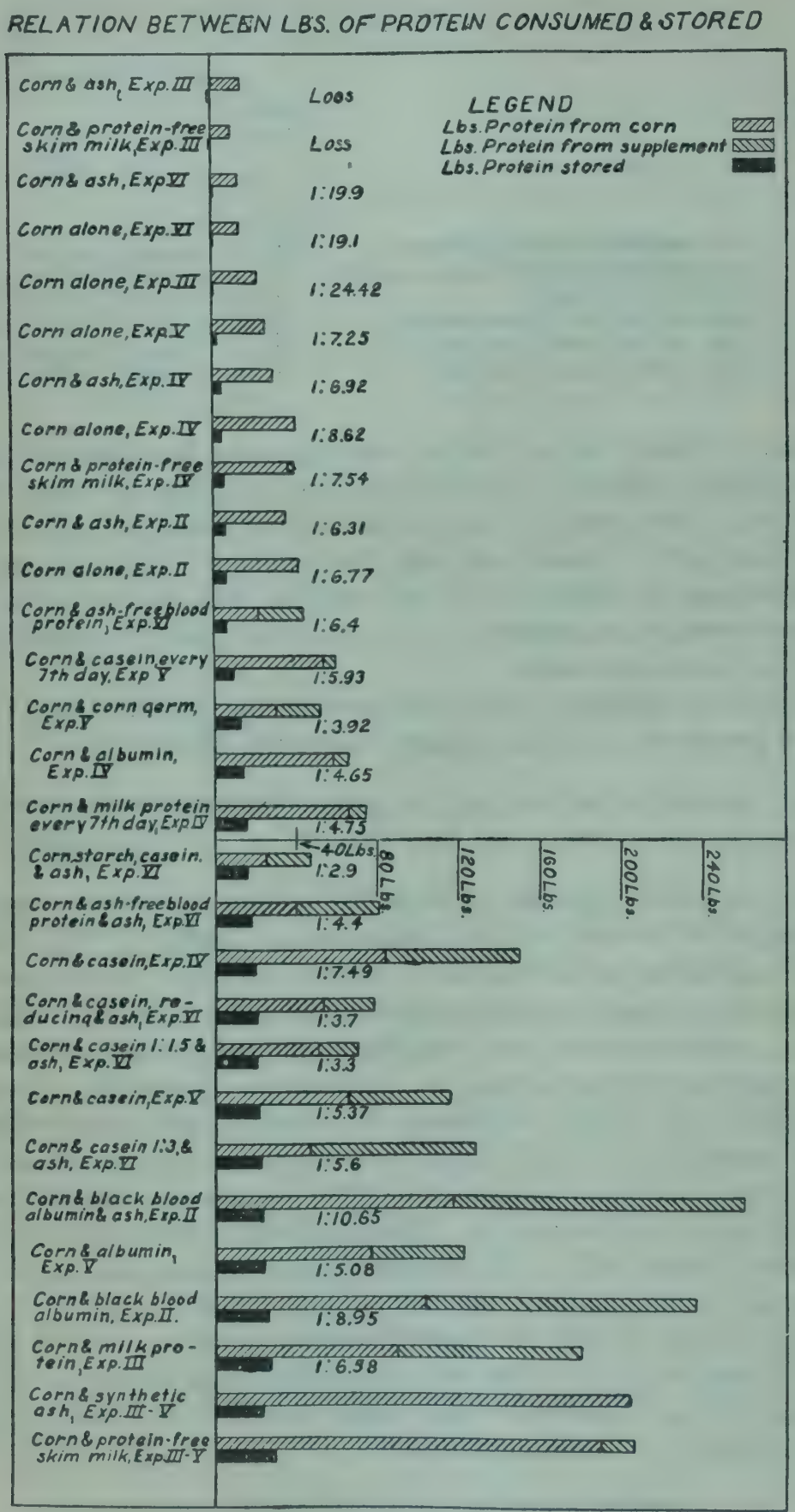

FIG. 15.—Graphical representation of the relation between pounds of protein consumed and pounds of protein stored.

RELATION BETWEEN PROTEIN CONSUMED AND THE SUM OF ASH, PROTEIN, AND FAT STORED

The pig is not an economical machine for the storage of protein. In the preceding pages it has been shown that the most narrow ratio in protein storage was 1 to 2.85. That is, 1 pound of protein was stored for nearly 3 pounds consumed. This pig stored 16.85 pounds of protein, though the ration contained 21.85 pounds of protein from casein and 24.84 pounds from corn. Protein from milk when fed in medium amounts was the most efficient. When other protein from animal sources was used, the ratio was wider. The pig which was fed 142 pounds of protein from black blood albumen and 118 pounds from corn stored only 24.27 pounds of protein, or less than 1 pound for more than 10 pounds consumed. The other principal constituent which the pig stores is fat. While protein can come only from the protein of the feed, the fat can come from the carbohydrates and the fat in the feed and also from the protein. The ash or mineral matter can come only from the ash of the feed.

The problem in this case is to compare the sum of ash, protein, and fat stored by each pig during the experiment with the amount of protein consumed. To what extent does the supplementary protein added to the corn stimulate the storage of these three constituents?

The five pigs noted above, two of which lost weight and the other of which gained only small amounts, will not be included in the comparisons which follow.

SUM OF CONSTITUENTS STORED FROM CORN FED ALONE OR WITH ASH

When pigs were fed corn alone they stored on the average 1 pound of protein for 7.5 pounds consumed. The digestible portion of the other 6.5 pounds of protein, together with the digestible fat and carbohydrates in the ration, were used for the production of energy and for the storage of fat. The amount of total constituents stored are presented in Table XIII, together with the figures on protein consumption.

TABLE XIII.—*Relation of ash plus protein plus fat stored to protein consumed*

Experiment No.	Ration.	Ash plus protein plus fat stored.	Protein consumed.		Ratio.
			From corn.	From supplement.	
II	Corn alone	58.46	42.58	.....	1.3:1
II	Corn and ash	46.83	36.07	.....	1.3:1
IV	Corn alone	44.09	41.87	.....	1.1:1
IV	Corn and ash	31.01	31.72	.....	1.0:1
IV	Corn and protein-free skim milk	60.70	37.17	3.24	1.5:1
V	Corn alone	26.55	27.25	.....	1.1:1
III–V	Corn and ash (continuation hog)	231.08	203.73	.....	1.1:1
III–V	Corn and protein-free skim milk	225.67	190.75	16.03	1.1:1

The average from these ratios is a little less than 1.1 to 1. That is, for 1 pound of protein consumed the total of ash, fat, and body protein stored is about 1.1 pounds. The older continuation pigs were using the food as efficiently as the younger pigs. As these two pigs did not start to make satisfactory gains till they were nearly 2 years old, it means that during the third or last year of their life the ratio was narrower than for the younger pigs.

SUM OF CONSTITUENTS STORED FROM CORN SUPPLEMENTED WITH A SMALL AMOUNT OF PROTEIN FROM MILK

The addition to corn of a small amount of protein from milk increased the amount of total constituents stored in ralation to the total protein consumed. This is shown in the figures given in Table XIV.

TABLE XIV.—*Relation of ash plus protein plus fat stored to protein consumed*

Experiment No.	Ration.	Ash plus protein plus fat stored.	Protein consumed.		Ratio.
			From corn.	From supplement.	
IV.....	Corn and milk protein every seventh day.....	119.68	66.60	8.00	1.61:1
IV.....	Corn and milk albumin.....	100.21	59.00	6.71	1.52:1
V.....	Corn and casein every seventh day...	75.70	55.16	5.61	1.25:1

The average of these ratios is very nearly 1.46 to 1, or 1.46 pounds of constituents stored for each pound of protein consumed, and shows the distinct advantage of adding this small amount of protein from milk.

SUM OF CONSTITUENTS STORED FROM CORN SUPPLEMENTED WITH A MEDIUM AMOUNT OF PROTEIN FROM MILK

When corn was supplemented with a medium amount of protein from milk amounting to a little less than one-third of the total protein fed, the ratio of constituents stored to protein consumed was 1.41 to 1. There were only two pigs fed with this medium amount of protein. (Table XV.)

TABLE XV.—*Relation of ash plus protein plus fat stored to protein consumed*

Experiment No.	Ration.	Ash plus protein plus fat stored.	Protein consumed.		Ratio.
			From corn.	From supplement.	
VI.....	Corn and casein reducing.....	109.08	51.03	20.81	1.52:1
VI.....	Corn and casein 1.5:1.....	103.21	53.07	25.84	1.31:1

Increasing the proportion of protein from milk from about one-tenth of the total to a little less than one-third of the total did not increase the sum of constituents stored for each pound of protein consumed.

SUM OF CONSTITUENTS STORED WHEN CORN WAS SUPPLEMENTED WITH LARGE AMOUNTS OF PROTEIN FROM MILK

The amount of protein storage was increased by adding a small amount of protein from milk, one-tenth of the total, to corn. This was further increased by adding more protein, a little less than one-third of the total. But the effectiveness could not be further increased by adding more protein. When the total constituents were considered, the effectiveness was still more limited. When the supplementary protein was about one-tenth of the total, it was more effective than when corn alone was used, but the effectiveness did not increase further. That the addition of large amounts of protein from milk does not increase the effectiveness over medium amounts is shown in Table XVI.

TABLE XVI.—*Relation of ash plus protein plus fat stored to protein consumed*

Experiment No.	Ration.	Ash plus protein plus fat stored.	Protein consumed.		Ratio.
			From corn.	From supplement.	
III	Milk protein (casein and albumin)	184.38	90.54	89.39	1.02:1
IV	Casein 1:3	149.97	84.65	66.65	1.00:1
V	do	119.25	65.72	50.72	1.03:1
V	Milk albumin	138.75	77.04	46.32	1.13:1
VI	Casein 1:3	106.68	47.35	80.70	.83:1

The average of these ratios is 1 to 1, or less than that obtained when corn was fed alone. When milk protein was about one-tenth of the total, the average was 1.46 to 1. When milk protein was about one-third of the total, the average was 1.41 to 1. Increasing the amount of supplementary protein beyond a certain amount will not enable the pig to store more constituents in proportion to the increase in protein added.

AMOUNT OF CONSTITUENTS STORED FROM CORN SUPPLEMENTED WITH LARGE AMOUNTS OF PROTEIN FROM VARIOUS SOURCES

That the total amount of constituents stored in proportion to the amount of protein consumed may be further decreased is shown by the figures given in Table XVII.

The protein from black blood albumen constituted over one-half of the total protein supplied, yet the total constituents stored when this large amount of protein was fed were less in proportion than with any other rations except the ash-free blood protein. The addition of ash to the black blood albumen did not increase its efficiency, but when ash

was added to the ash-free blood protein, the efficiency was greatly increased. The protein from corn germ was as efficient in its effect on the storage of total nutrients as the seventh day feeding of casein. The corn, starch, and casein ration was the most efficient of all the rations tried. About 1⅔ pounds of constituents were stored for each pound of protein consumed. It was shown above that this ration was also the most efficient in relation to protein storage.

TABLE XVII.—*Relation of ash plus protein plus fat stored to protein consumed*

Experiment No.	Ration.	Ash plus protein plus fat stored.	Protein consumed.		Ratio.
			From corn.	From supplement.	
II	Corn and black blood albumen	229.59	105.97	129.21	0.98:1
II	Corn, black blood albumen, and ash	244.21	118.20	142.25	.94:1
V	Corn and corn germ	65.71	31.07	21.47	1.25:1
VI	Corn and ash-free blood protein	31.50	22.27	23.61	.69:1
VI	Corn, ash-free blood protein, and ash	82.85	39.29	41.66	1.03:1
VI	Corn, starch, casein, and ash	78.25	24.84	22.61	1.66:1

GRAPHIC PRESENTATION OF THE RELATION BETWEEN THE PROTEIN CONSUMED AND THE TOTAL GAIN OF CHEMICAL CONSTITUENTS

The relation between the protein consumed and the storage of ash, protein, and fat is shown graphically in figure 16. The bars are arranged in order of the increasing amounts of constituents stored.

The efficiency of corn alone as a ration for growing pigs, as measured by the total constituents stored, can be greatly increased by the addition of a small amount of protein from milk. If the amount of protein from milk is increased above a certain amount, the efficiency of the ration as a whole is decreased. The increase in protein simply increased the rate of growth. This is true when the ratio is narrow. The proteins from blood were the least efficient although they furnished one-half of the total protein. Proteins from milk were the most efficient when fed in a ratio made wide by the addition of starch. The proteins from corn germ, as measured by the total constituents stored, compare well with the proteins from other sources.

INFLUENCE OF QUANTITY OF PROTEIN CONSUMED UPON QUANTITY OF FAT STORED

In the preceding discussion it has been shown that the quantity and quality of protein consumed has a limited influence on the relative quantity of the total ash, protein, and fat stored. On the whole, the more protein that is consumed in the ration the more constituents will be stored; but a ration that is liberal in protein will not store a pro-

portionately larger quantity of these constituents as compared with one that is relatively poor. When corn was the exclusive source of food, the quantity of constituents stored was only 1.1 pounds for each pound of protein consumed. This was increased to 1.46 and 1.66 pounds when milk protein was used in a ration whose nutritive ratio was wide. But

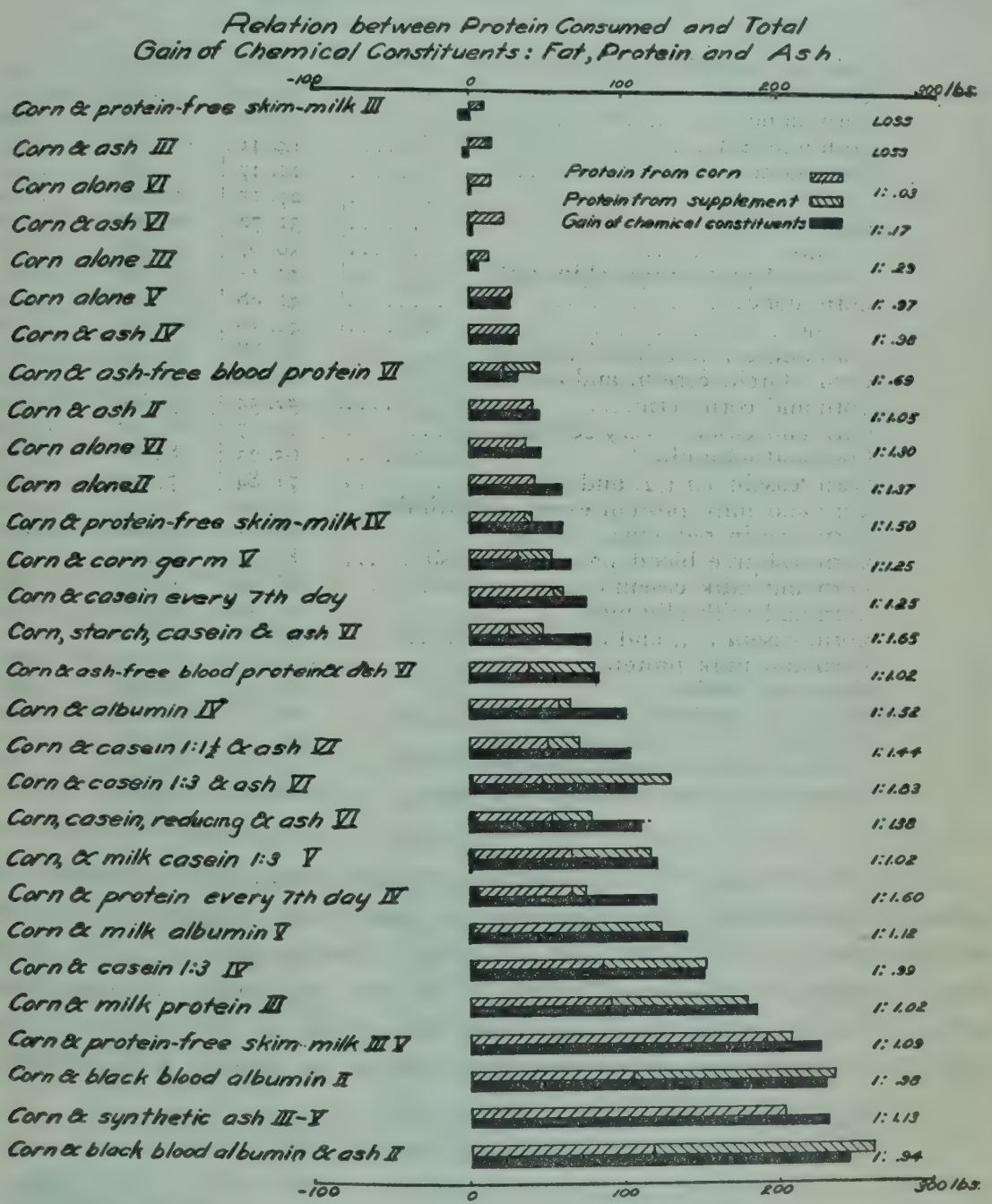

FIG. 16.—Graphical representation showing the relation between pounds of protein consumed and total pounds of protein, ash, and fat stored.

when large amounts of protein were used the total constituents stored were about 1 pound for each pound of protein consumed.

The pig stores fat in larger quantities than any other constituent, except when of small size. In small pigs moisture is the largest constituent. In pigs of 200 pounds weight or over, fat is by far the largest constituent.

In Table XVIII are given the pounds of protein consumed, the pounds of fat stored, and the ratio of protein consumed to fat stored.

TABLE XVIII.—*Influence of protein consumption on fat storage*

Experiment No.	Ration.	Protein consumed.	Fat stored.	Ratio.
III	Corn and protein-free skim milk	9.27	−6.22	Loss.
VI	Corn and ash	12.36	1.35	0.10:1
VI	Corn alone	12.99	−.58	Loss.
III	Corn and ash	14.41	−2.86	Loss.
III	Corn alone	22.47	6.55	.29:1
V	do	27.25	22.08	.81:1
IV	Corn and ash	31.72	26.05	.82:1
II	do	36.07	39.83	1.10:1
IV	Corn and protein-free skim milk	40.41	53.91	1.33:1
IV	Corn alone	41.88	38.90	.93:1
II	do	42.58	51.02	1.20:1
VI	Corn and ash-free blood protein	45.88	23.48	.51:1
VI	Corn, starch, casein, and ash	47.45	58.29	1.23:1
V	Corn and corn germ	52.54	50.48	.96:1
V	Corn and casein every seventh day	60.77	64.02	1.05:1
IV	Corn and albumin	65.75	84.86	1.29:1
VI	Corn, casein 1:1 1/2, and ash	71.84	77.35	1.08:1
IV	Corn and milk protein every seventh day	74.60	102.37	1.37:1
VI	Corn, casein reducing, and ash	78.91	83.08	1.05:1
VI	Corn, ash-free blood protein, and ash	80.95	60.96	.75:1
V	Corn and milk casein 1:3	116.44	93.95	.80:1
V	Corn and milk albumin	123.36	110.18	.89:1
VI	Corn, casein 1:3, and ash	128.05	78.82	.61:1
III	Corn and milk protein	179.93	153.30	.85:1
IV	Corn and casein 1:3	151.30	126.61	.84:1
III–V	Corn and synthetic ash	203.73	201.34	.99:1
III–V	Corn and protein-free skim milk	206.78	189.69	.92:1
II	Corn and black blood albumen	235.48	199.80	.85:1
II	Corn, black blood albumen, and ash	260.45	215.03	.82:1

When the five pigs which either lost weight during the experiment or gained an abnormally small amount of weight are omitted, the results on the other 24 pigs show the following: Nine of the 24 stored more than 1 pound of fat for each pound of protein consumed. Among these 9 the 4 which stored the largest amount of fat in proportion to the amount of protein consumed were the pigs fed as stated in Table XIX.

TABLE XIX.—*The nutritive ratio and ratio of protein to fats stored*

Ration.	Nutritive ratio.	Ratio of protein consumed to fat stored.
Corn, starch, casein and ash	1 : 8.76	1 : 1.23
Corn and milk protein every seventh day	1 : 7.64	1 : 1.37
Corn and milk albumin	1 : 7.71	1 : 1.29
Corn and protein-free skim milk	1 : 9.44	1 : 1.33

These were all wide nutritive ratios. The pigs which stored the smallest amount of fat in proportion to the amount of protein fed were

the pigs fed the corn and ash-free blood protein and corn and casein in experiment VI. These had a nutritive ratio of 1 to 4 and 1 to 3.34, respectively.

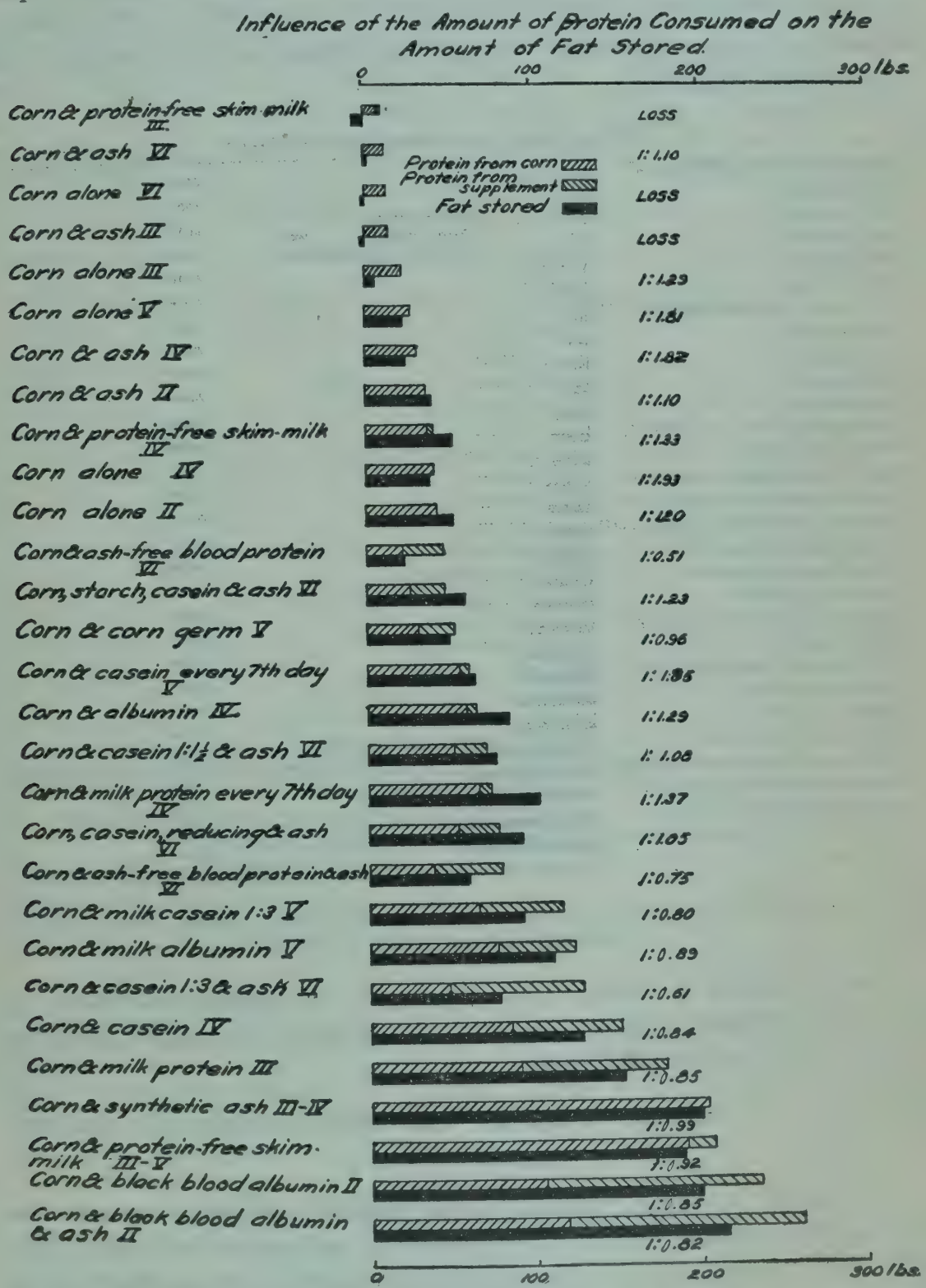
Influence of the Amount of Protein Consumed on the Amount of Fat Stored.
0
100
200
300 lbs.
Protein from corn
Protein from supplement
Fat stored
Corn & protein-free skim-milk III. Loss
Corn & ash VI 1:1.10
Corn alone VI Loss
Corn & ash III Loss
Corn alone III 1:1.23
Corn alone V 1:1.81
Corn & ash IV 1:1.82
Corn & ash II 1:1.10
Corn & protein-free skim-milk IV 1:1.33
Corn alone IV 1:1.93
Corn alone II 1:1.20
Corn & ash-free blood protein VI 1:0.51
Corn, starch, casein & ash VI 1:1.23
Corn & corn germ V 1:0.96
Corn & casein every 7th day V 1:1.85
Corn & albumin IV 1:1.29
Corn & casein 1:1½ & ash VI 1:1.08
Corn & milk protein every 7th day IV 1:1.37
Corn, casein, reducing & ash VI 1:1.05
Corn & ash-free blood protein & ash VI 1:0.75
Corn & milk casein 1:3 V 1:0.80
Corn & milk albumin V 1:0.89
Corn & casein 1:3 & ash VI 1:0.61
Corn & casein IV 1:0.84
Corn & milk protein III 1:0.85
Corn & synthetic ash III-IV 1:0.99
Corn & protein-free skim-milk III-V 1:0.92
Corn & black blood albumin II 1:0.85
Corn & black blood albumin & ash II 1:0.82
0
100
200
300 lbs.

FIG. 17.—Graphical representation of the relation between the amount of protein consumed and the amount of fat stored.

Most of the other pigs stored from 0.8 pound to 1 pound of fat for each pound of protein consumed. The source of protein did not seem to have any distinct influence. The pigs fed on corn and black blood

albumin stored, on the whole, as much fat in proportion to the protein consumed as the pigs fed protein from milk. The two old continuation pigs, one being fed on corn and ash and the other on corn and protein-free skim milk, stored as much fat in proportion to the amount of protein consumed as the younger pigs which received protein from sources other than corn.

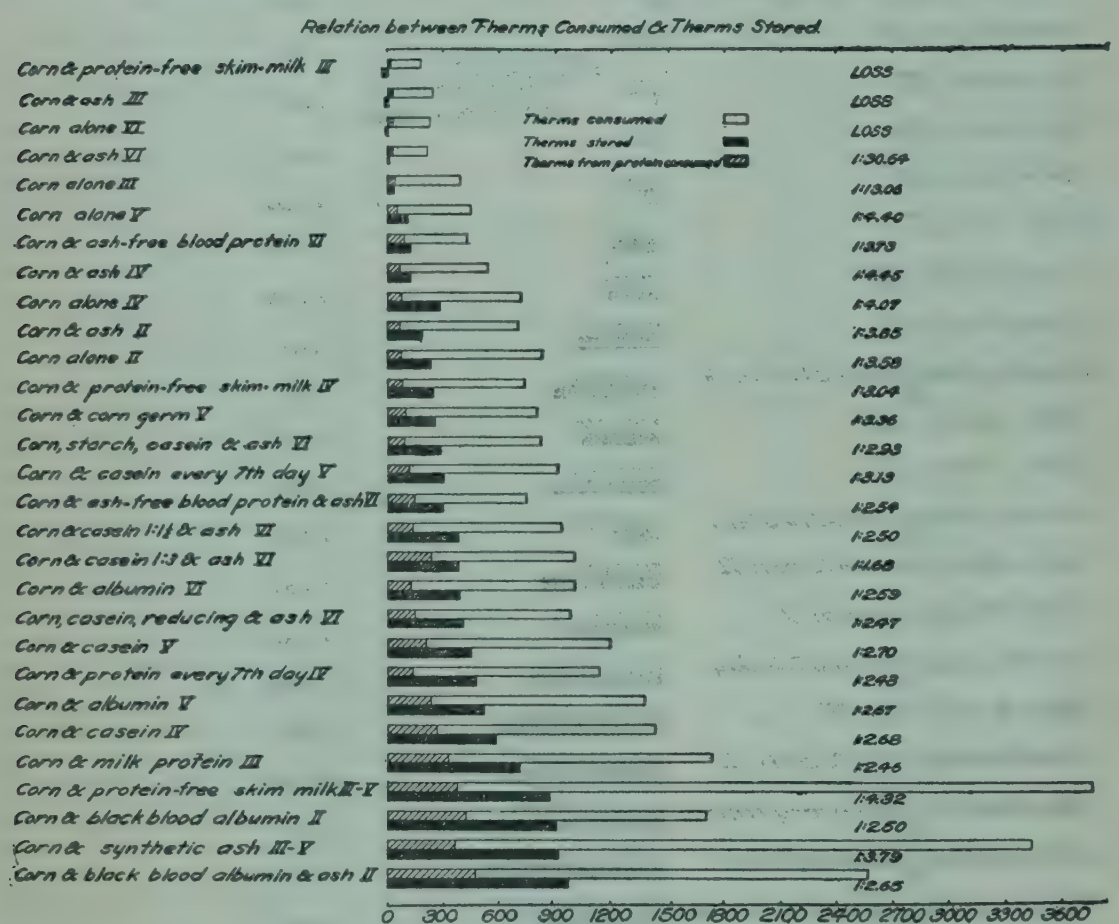

FIG. 18.—Graphical representation of the relation between total therms consumed, therms from protein consumed, and the total therms stored.

The facts given in Table XVIII are graphically presented in figure 17. The bars representing the protein consumed are a little taller than the bars representing the fat stored.

QUANTITATIVE RELATION BETWEEN THE THERMS CONSUMED AND THE THERMS STORED

The therms stored in relation to the therms consumed measure the total effectiveness of the ration. Protein stored in the body can come only from the protein in the food. The digestible fat and carbohydrates of the food are used for the production of energy and for fat storage. The two principal constituents stored in the body are protein and fat. Any animal fed for the purpose of food production is valuable in the proportion in which it stores protein and fat relative to the amount of carbohydrates, protein, and fat consumed.

The figures giving the total thermal value of the feeds consumed in the different rations during the feeding trial, as well as the total thermal value of the protein and fat stored, are shown in Table XX. The thermal value of the ration was calculated by multiplying the pounds of carbohydrates and protein consumed by 1.8 and the pounds of ether extract by 4.05, assuming that the energy value of these constituents is equal to 1,800 calories per pound of carbohydrates and protein and 4,050 calories per pound of ether extract. For the constituents stored the figures 1.86 and 4.2 were used for the respective constituents. The ratio was calculated by dividing the therms stored into the therms consumed. The graphic representation of these various values is shown in figure 18.

TABLE XX.—*Relation between therms consumed and therms stored*

Experiment No.	Ration.	Therms stored.	Therms consumed.	Ratio.
III	Corn and protein-free skim milk	−26.82	176.43	Loss.
III	Corn and ash	−13.23	250.13	Loss.
VI	Corn alone	−1.23	223.83	Loss.
VI	Corn and ash	6.96	212.92	30.64 : 1
V	Corn alone	101.94	448.75	4.40 : 1
III	do	29.88	389.98	13.06 : 1
VI	Corn and ash-free blood protein	114.26	426.51	3.73 : 1
IV	Corn and ash	120.53	535.81	4.45 : 1
IV	Corn alone	176.31	707.47	4.07 : 1
II	Corn and ash	181.87	700.57	3.85 : 1
II	Corn alone	231.10	826.90	3.58 : 1
IV	Corn and protein-free skim milk	241.78	735.47	3.04 : 1
V	Corn and corn germ	241.91	812.98	3.36 : 1
VI	Corn, starch, casein, and ash	281.62	825.07	2.93 : 1
V	Corn and casein every seventh day	294.37	922.34	3.13 : 1
VI	Corn, ash-free blood protein, and ash	296.05	752.61	2.54 : 1
VI	Corn and casein 1 : 1½ and ash	372.54	932.27	2.50 : 1
VI	Corn, casein 1 : 3, and ash	381.52	1,020.66	2.68 : 1
IV	Corn and albumin	391.20	1,012.09	2.59 : 1
VI	Corn, casein, reducing, and ash	397.14	980.02	2.47 : 1
V	Corn and casein	444.61	1,200.27	2.70 : 1
IV	Corn and milk protein every seventh day	469.41	1,140.43	2.43 : 1
V	Corn and albumin	518.97	1,387.45	2.67 : 1
IV	Corn and casein	581.96	1,558.12	2.68 : 1
III	Corn and milk protein	710.02	1,746.15	2.46 : 1
III–V	Corn and protein-free skim milk	871.00	3,766.56	4.32 : 1
II	Corn and black-blood albumen	908.02	2,307.81	2.54 : 1
III–V	Corn and synthetic ash	911.57	3,457.76	3.79 : 1
II	Corn, black-blood albumen, and ash	970.14	2,569.86	2.65 : 1

CORN WITHOUT ADDITIONAL PROTEIN [1]

The pigs fed on corn alone or on corn and ash or on corn and protein-free skim milk stored less therms on the average in proportion to the thermal value of the feeds consumed than did the pigs to whose ration protein from animal sources was added. The average ration for all the pigs which had corn and had no protein from other sources was nearly 1 to 4.

[1] The five pigs which made a very small gain are not included in discussion.

That is, for four therms consumed in the ration only one was stored in the form of protein or fat. The two continuation pigs stored energy in as efficient a ratio as the younger hogs. Since these two pigs did not make material gains for the first year of the experiment, it means that they must have stored energy in a more efficient ratio during the last year.

CORN WITH ADDITIONAL PROTEIN [1]

The ratio for the pig fed corn and corn germ was 1 to 3.36. This was wider than for any of the pigs fed additional protein, but narrower than for any pig fed corn alone. For the pig fed casein every seventh day the ratio was 1 to 3.13—wider than for any other pig fed protein from milk in addition to corn. For the pig fed milk protein every seventh day the ratio was 1 to 2.43. This was the narrowest ratio for any pig. For the two pigs fed milk protein in medium amounts, one corn and casein 1 to 1.5 and the other corn and casein, reducing, the ratios were 1 to 2.50 and 1 to 2.47, respectively, or narrower than for any of the pigs fed milk protein in the larger amounts. The average ratio for the four pigs fed milk protein in large amounts was 1 to 2.63. From the standpoint of energy storage this larger feeding of milk protein was not as efficient as the smaller feeding of milk protein. The ratio for the pig fed corn, starch, casein, and ash was 1 to 2.93. This was wider than for any pig fed milk protein in addition to corn, except one fed casein every seventh day. For the two pigs fed black-blood albumen the ratio was 1 to 2.58. In this case the ash had no influence. With the ash-free blood protein the addition of ash was a distinct advantage. Without the ash the ratio was 1 to 4.32, as wide as for corn alone. With ash the ratio was 1 to 2.54, as narrow as when large amounts of milk protein were fed.

This analysis shows that feeding corn alone is not advantageous from the standpoint of energy stored. It also shows the distinct advantage of adding a small amount of protein from other sources. The pigs to whose ration from one-tenth to one-third of the total protein in the ration was from milk stored energy in as large a ratio as those whose ration contained over half the protein from milk or from other animal sources.

CONCLUSIONS

PROTEIN STORAGE

1. Pigs fed on corn alone store on the average 1 pound of protein for 7.5 pounds of protein consumed, provided that before the pigs are put on the ration they have attained a certain development.

2. When corn was supplemented with small amounts of protein from milk, from one-eighth to one-tenth of the total protein in the ration, the pigs stored 1 pound of protein for 5.11 pounds consumed.

[1] The five pigs which made a very small gain are not included in the discussion.

3. When corn was supplemented with a medium amount of protein from milk, amounting to nearly one-third of the total protein in the ration, the pigs stored 1 pound of protein for 3.5 pounds consumed.

4. When corn was supplemented with a large amount of protein from milk, from one-half to almost two-thirds of the total protein in the ration, the pigs stored on the average 1 pound of protein for 6 pounds consumed. The amount of protein stored varied from one-fourth to one-half the amount in the milk proteins consumed.

5. A large amount of protein from other sources than corn produces a more rapid rate of gain, but at the expense of the efficiency of protein storage.

6. The most efficient ration from the standpoint of protein storage is one which contains a small amount of protein from milk so combined with other feeds as to make a wide nutritive ratio.

ASH, FAT, AND PROTEIN STORAGE

7. When corn was fed alone the sum of ash, fat, and body protein stored was 1.1 pounds for each pound of protein consumed.

8. When corn was supplemented with a small amount of protein from milk, the sum of ash, fat, and body protein stored was 1.46 pounds for each pound of protein consumed.

9. When corn was supplemented with a medium amount of protein from milk the sum of ash, fat, and body protein stored was 1.41 pounds for each pound of protein consumed.

10. When corn was supplemented with a large amount of protein from milk or other animal proteins the sum of ash, fat, and body protein stored was less than 1.1 pounds for each pound of protein consumed, a less efficient storage of nutrients than when corn was fed alone. This statement leaves rate of growth out of consideration.

11. Feeds combined so as to make a wide nutritive ratio produced the largest amount of storage of ash, fat, and body protein in relation to the amount of protein in the feed.

FAT STORAGE

12. Nine of the 24 pigs studied in these experiments stored more than 1 pound of fat for each pound of protein consumed. Among these 9 the 4 which stored fat in largest amount in proportion to the amount of protein consumed were fed rations having a wide nutritive ratio.

13. Most of the 24 pigs studied in these experiments stored 0.8 pound to 1 pound of fat for each pound of protein consumed. The source of protein did not seem to have any distinct influence. The protein from black blood albumen was as efficient for fat storage as the protein from milk. The 2 pigs fed till they were nearly 3 years old, and whose sole source of protein was corn, stored fat as efficiently in proportion to the amount of protein consumed as the younger pigs which received protein from other sources than corn.

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ENERGY STORAGE

14. Pigs whose sole source of protein was corn stored less therms on the average in proportion to the thermal value of the feeds consumed than did the pigs to whose ration protein from animal sources was added.

15. From the standpoint of energy storage the small amounts of milk protein were as efficient as the larger amounts, but not more so.

16. From the standpoint of energy storage the feeding of corn alone is not efficient. The addition of a small amount of protein from other sources from one-tenth to one-third of the total is as efficient as when one-half or more of the protein comes from other sources.

IV. EFFECT OF PROLONGED FEEDING OF CORN ALONE, CORN AND ASH, OR CORN AND PROTEIN-FREE SKIM MILK ON THE GROWTH AND DEVELOPMENT OF PIGS

That corn alone, corn and ash, or corn and protein-free skim milk is deficient as a ration for young growing pigs has been shown in section III. In experiment II of the series of experiments from which these data have been taken, one of the pigs fed corn alone was continued on this feed for 1,060 days, counting from the beginning of the experiment. This pig was 4 ½ months old at the beginning of the trial, and weighed 54 pounds. In experiment III of this same series, one pig from the lot fed corn and ash and one from the lot fed corn and protein-free skim milk were continued on through for 900 days, counting from the beginning of the experiment. These two pigs were 3½ months old at the beginning of the experiment. The pig fed corn and ash weighed 42 pounds and the pig fed corn and protein-free skim milk weighed 47 pounds at the beginning of the experiment. These ages and weights are important because if these pigs had been younger at the beginning of the trial the results might have been different.

GAIN IN LIVE WEIGHT

The live weight of these pigs at 100-day periods, the gain for these periods, and the corn consumed are given in Table XXI.

The pig fed corn and ash did not make constant gains until 400 days after the beginning of the experiment. The total gain in the first 400 days of the experiment was only 18 pounds, and this was all gained in the first 200 days. This net gain of 18 pounds was at the expense of consuming 361.3 pounds of corn, or 20 pounds consumed for 1 pound of gain. During the last 523 days of the trial the gain was 334 pounds and the corn consumed was 1,646 pounds, or 1 pound gain for 5 pounds consumed. The period of greatest increase was from the seven hundredth to the nine hundredth day. In this time the consumption was 944 pounds and the gain 215 pounds, or 1 pound gain for 4.4 pounds consumed.

TABLE XXI.—*Live weight, gain, and total feed consumed, by 100-day periods*

PIG FED CORN AND ASH

Period.	Weight.	Gain.	Total corn consumed.
Days.	*Pounds.*	*Pounds.*	*Pounds.*
0 to 100 [a]	56	14	121. 50
100 to 200	65	9	69. 70
200 to 300	53	−12	72. 40
300 to 400	60	7	97. 70
400 to 500	87	27	143. 45
500 to 600	131	44	236. 00
600 to 700	168	37	228. 00
700 to 800	269	101	383. 80
800 to 900	383	114	560. 60
900 to 923	394	7	112. 05
Total			2, 007. 20

PIG FED CORN AND PROTEIN-FREE SKIM MILK

0 to 100 [b]	51	4	81. 45
100 to 200	56	5	78. 30
200 to 300	54	−2	82. 00
300 to 400	64	10	104. 60
400 to 500	89	25	149. 05
500 to 600	132	43	224. 60
600 to 700	202	60	220. 00
700 to 800	293	91	409. 00
800 to 900	379	86	425. 65
900 to 923	375	−4	121. 40

PIG FED CORN ALONE

0 to 100 [c]	58	4	93. 99
100 to 200	68	10	111. 47
200 to 300	82	14	121. 00
300 to 400	128	36	201. 40
400 to 500	134	6	239. 10
500 to 600	135	1	192. 00
600 to 700	180	45	200. 00
700 to 800	243	63	329. 90
800 to 900	370	127	548. 80
900 to 1,000	481	111	676. 00
1,000–1,060	596	115	386. 00

[a] In the 3½ months before the experiment began the pig attained a weight of 42 pounds.
[b] In the 3½ months before the experiment began the pig attained a weight of 47 pounds.
[c] In the 4½ months before the experiment began the pig attained a weight of 54 pounds.

The pig fed corn and protein-free skim milk gained 17 pounds in the first 400 days of the trial and consumed 346 pounds of corn, or 20 pounds for each pound gain. In the last 523 days of the trial he gained 311 pounds and consumed 1,552 pounds of corn, 5 pounds for each pound of gain.

This pig also made his most rapid gains in the last 200 days, gaining 177 pounds. In this time he consumed 834 pounds of corn and made

1 pound gain for 4.7 pounds consumed. The corn and protein-free skim milk ration had no advantage over the corn and ash ration. The pig fed corn alone did not make steady and constant gains till after the six hundredth day of the trial. He had gained in this time 81 pounds and consumed 959 pounds of corn, or 1 pound of gain for 11.8 pounds con-

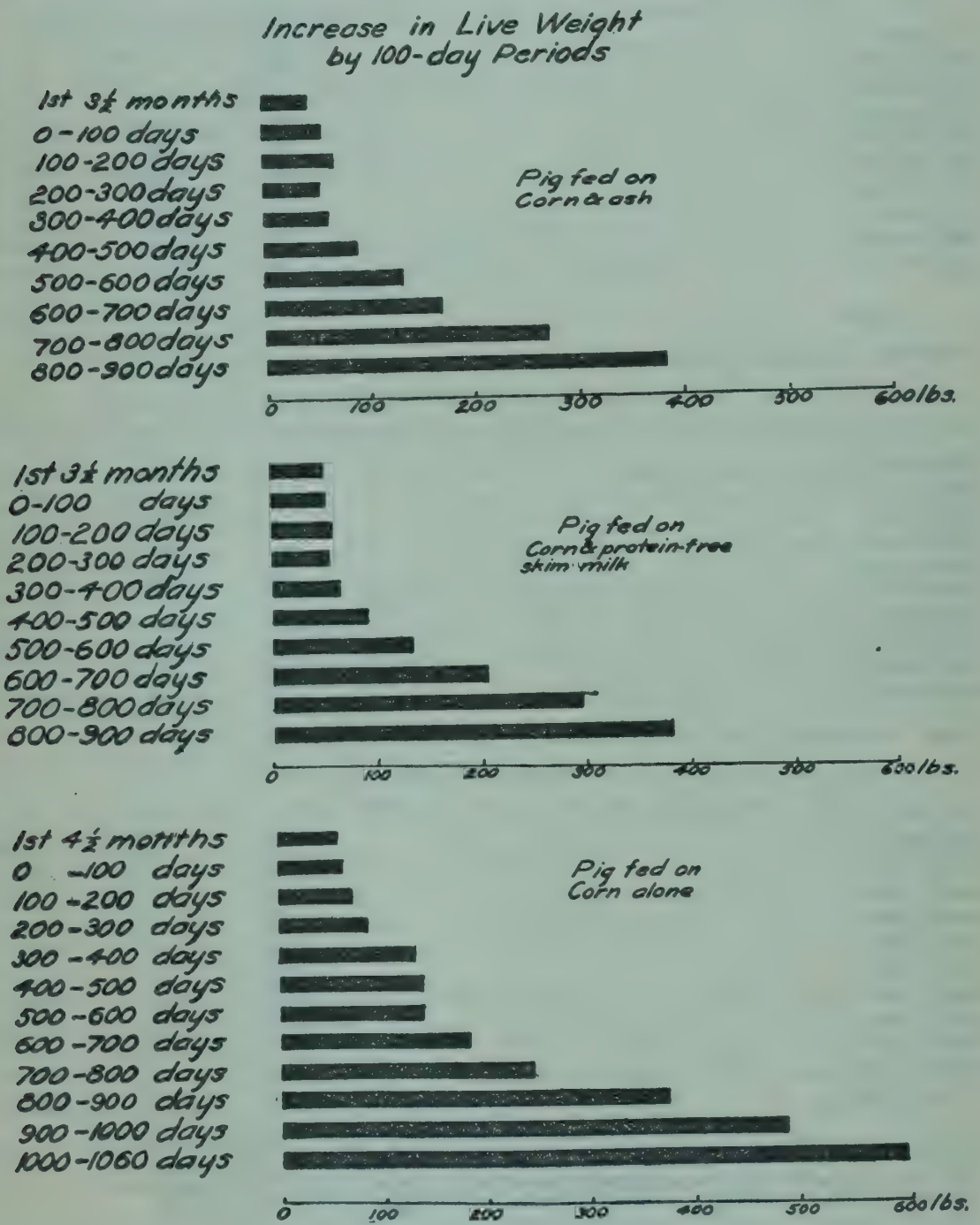

FIG. 19.—Graphical representation of the increase in live weight by 100-day periods.

sumed. This is a better record than for the other two pigs. This gain took place mostly in the first 400 days. In this time the gain was 74 pounds and the consumption was 428 pounds, or 1 pound of gain for 5.7 pounds consumed. There was a period from the four hundredth to the six hundredth day that there was very little gain. In the last 460

days the gain was 461 pounds and the corn consumed was 2,141 pounds, or 1 pound of gain for 4.6 pounds consumed. In the last period of his life the pig fed corn alone made as good gains as the pig fed corn and ash or corn and protein-free skim milk. The facts presented above are graphically shown in figure 19.

The outstanding fact is that in all these pigs there was a long period in which the gains were very small, and after this period the rate of gain and the gain for the amount consumed was equal to that of pigs fed a standard ration. It evidently means that before a pig can make satisfactory gains it must attain a certain development. When corn alone is the sole diet this development is attained very slowly. When alfalfa pasture was the sole source of food, this development took place more rapidly, although the pigs did not gain much in weight. This fact was illustrated by some pigs in this series of experiments which were fed on alfalfa pasture alone. One lot of those pigs gained an average of only 3 pounds each in 120 days. When they were put on corn alone they gained 67 pounds in as many days. This gain was produced from 284 pounds of corn, or 4¼ pounds of corn for each pound of gain. One pig from this lot was analyzed when taken off the alfalfa pasture. Compared with a control pig killed at the beginning of the experiment, he had gained 11.66 pounds moisture, 0.77 pound ash, 2.93 pounds protein, but lost 14.84 pounds fat. Unfortunately no pig from this lot was slaughtered at the end of the trial. But, as has been shown in section I, it is safe to compare pigs of approximately equal size for the purpose of calculating chemical composition. A comparison of the pig of this lot fed corn alone after alfalfa pasture with a slaughtered pig fed corn alone, showed that the following average pounds of gain were made on corn alone in 67 days: moisture, 11.73; ash, 0.87; protein, 3.88; fat, 56.71. While the gains on alfalfa pasture were unsatisfactory, the pigs had attained a certain development of body which gave them vigor to make rapid gains when put on corn alone. It should be noted that the gains were comparatively large in protein and ash on alfalfa pasture, and on corn alone the gain was largely fat. Alfalfa alone is strong in those growth-producing properties in which corn is weak. Corn is strong in fat-producing properties in which alfalfa is weak. Hughes (*6*) has shown that when chickens are fed on corn alone, the growth curve is similar to the one made by pigs fed on corn alone. He also shows that the rate of growth in chickens can be increased by forced feeding.

NUTRIENTS AND THERMS IN CORN CONSUMED

The average composition of corn fed these pigs was assumed to be the same as the average of the corn used in all these experiments—namely, protein, 9.65 per cent; carbohydrates, 75 per cent; fat, 3.8 per cent. In

making calculations for thermal value, 1,800 calories were used for each pound of carbohydrate and protein and 4,060 calories for each pound of fat. On the basis of these figures of thermal value, composition, and the amount of corn consumed in the different 100-day periods, the total therms in corn consumed were calculated. The small thermal value in the organic matter of the protein-free skim milk was omitted from these figures. The figures as obtained are found in Table XXII. The chief value of these figures is to obtain data to use in calculations given further on.

TABLE XXII.—*Weight of nutrients and therms in corn consumed*

PIG FED CORN AND ASH

Period.	Total corn consumed.	Carbohydrates in corn.	Therms in carbohydrates.	Fat in corn.	Therms in fat.	Protein in corn.	Therms in protein.	Total therms in corn.
Days.	*Pounds.*	*Pounds.*		*Pounds.*		*Pounds.*		
0 to 100 [a]	121.50	91.13	164.03	4.62	18.76	11.72	21.10	213.89
100 to 200	69.70	52.28	94.10	2.65	10.75	6.73	12.11	116.96
200 to 300	72.40	54.30	97.74	2.75	11.17	6.99	12.58	121.49
300 to 400	97.70	73.28	131.90	3.71	15.06	9.43	16.97	163.93
400 to 500	143.45	107.59	193.66	5.45	22.12	13.84	24.91	240.69
500 to 600	236.00	177.00	318.60	8.97	36.41	22.77	40.99	396.00
600 to 700	228.00	171.00	307.80	8.66	35.16	22.00	39.60	382.56
700 to 800	383.80	287.85	518.13	14.58	59.19	37.04	66.67	643.99
800 to 900	560.60	420.45	756.81	21.30	86.48	54.10	97.38	940.67
900 to 923	112.05	84.04	151.27	4.26	17.30	10.81	19.46	188.03
Total	2,007.20					195.43		

PIG FED CORN AND PROTEIN-FREE SKIM MILK

0 to 100 [a]	81.45	61.09	109.96	3.10	12.59	7.86	14.15	136.70
100 to 200	78.30	58.73	105.71	2.98	12.10	7.56	13.61	131.42
200 to 300	82.00	61.50	110.70	3.12	12.67	7.91	14.24	137.61
300 to 400	104.60	78.45	141.21	3.97	16.12	10.09	18.16	175.49
400 to 500	149.05	111.79	201.22	5.66	22.98	14.38	25.88	250.08
500 to 600	224.60	168.45	303.21	8.53	34.63	21.67	39.01	376.85
600 to 700	220.00	165.00	297.00	8.36	33.94	21.23	38.21	369.15
700 to 800	409.00	306.75	552.15	15.54	63.09	39.47	71.05	686.29
800 to 900	425.65	319.24	574.63	16.17	65.65	41.08	73.94	714.22
900 to 923	121.40	91.05	163.89	4.61	18.72	11.72	21.10	203.71
Total	1,898.05					182.97		

PIG FED CORN ALONE

0 to 100 [b]	93.99	70.49	126.88	3.57	14.49	9.07	16.33	157.70
100 to 200	111.47	83.60	150.48	4.24	17.21	10.75	19.35	187.04
200 to 300	121.00	90.75	163.35	4.60	18.68	11.68	21.02	203.05
300 to 400	201.40	151.05	271.89	7.65	31.06	19.44	34.99	337.94
400 to 500	239.10	179.33	322.79	9.09	36.91	23.07	41.53	401.23
500 to 600	192.00	144.00	259.20	7.30	29.63	18.53	33.35	322.18
600 to 700	200.00	150.00	270.00	7.60	30.86	19.30	34.74	335.60
700 to 800	329.90	247.43	445.37	13.54	50.91	31.84	57.31	553.59
800 to 900	548.80	411.60	740.88	20.85	84.65	52.96	95.33	920.86
900 to 1,000	676.00	507.00	912.60	25.69	104.30	65.23	117.41	1,134.31
1,000 to 1,060	386.00	289.50	521.10	14.67	59.56	37.25	67.05	647.71
Total	3,099.66					299.12		

[a] No data for 3½ months preceding experiment.
[b] No data for 4½ months preceding experiment.

TABLE XXIII.—*Protein stored and thermal value*

PIG FED CORN AND ASH

Period.	Live weight.	Percentage of protein.	Total protein in body.	Protein stored.	Therms in protein stored.
Days.	*Pounds.*		*Pounds.*	*Pounds.*	
0 to 100 [a]	56	11	6.16	1.54	2.86
100 to 200	65	11	7.15	.99	1.84
200 to 300	53	11	5.83	−1.32	−2.46
300 to 400	60	11	6.60	.77	1.43
400 to 500	87	11	9.57	2.97	5.52
500 to 600	131	10	13.10	3.53	6.57
600 to 700	168	10	16.80	3.70	6.88
700 to 800	269	9	24.21	7.41	14.15
800 to 900	383	9	34.47	10.26	18.17
900 to 923	394	9	35.46	.99	1.84
Total				32.16	

PIG FED CORN AND PROTEIN-FREE SKIM MILK

0 to 100 [b]	51	11	5.61	0.44	0.81
100 to 200	56	11	6.16	.55	1.02
200 to 300	54	11	5.94	−.22	−.41
300 to 400	64	11	7.04	1.10	2.05
400 to 500	89	11	9.79	2.75	5.12
500 to 600	132	10	13.20	3.41	6.34
600 to 700	202	10	20.20	7.00	13.02
700 to 800	293	9	26.37	6.17	11.48
800 to 900	379	9	34.11	7.74	7.05
900 to 923	375	9	33.75	−.36	−.67
Total				28.16	

PIG FED CORN ALONE

0 to 100 [c]	58	11	6.38	0.44	0.82
100 to 200	68	11	7.48	1.10	1.05
200 to 300	82	11	9.02	1.54	2.86
300 to 400	128	10	12.80	3.78	7.03
400 to 500	134	10	13.40	.60	1.12
500 to 600	135	10	13.50	.10	.19
600 to 700	180	10	18.00	4.50	8.37
700 to 800	243	9	21.87	3.87	7.20
800 to 900	370	9	33.30	11.43	21.26
900 to 1,000	481	9	43.29	9.99	18.58
1,000 to 1,060	596	9	53.64	10.35	19.25
Total				47.70	

[a] In the 3½ months before the experiment began the pig attained a weight of 42 pounds, of which 11 per cent was protein. The total protein in the body was 4.62 pounds.

[b] In the 3½ months before the experiment began the pig attained a weight of 47 pounds, of which 11 per cent was protein. The total protein in the body was 5.17 pounds.

[c] In the 4½ months before the experiment began the pig attained a weight of 54 pounds, of which 11 per cent was protein. The total protein in the body was 5.94 pounds.

PROTEIN STORAGE

In calculations of the pounds of protein stored in the body it was assumed that the percentage of protein on the basis of live weight was 11 for weights less than 100 pounds, 10 for weights between 100 and 200 pounds, and 9 for weights above 200 pounds. These assumptions are based on average composition of pigs of similar weights. As the last period, 900 to 923 days, for two of the pigs is very short, the figures for this period should not be averaged or considered of any great significance. For calculating the thermal value of the protein stored, 1,860 calories per pound were used. This is not the total thermal value of the protein, but the thermal value of protein used as food. The figures as obtained are given in Table XXIII. They show that in the first 400 days of the experiment about 2 pounds of protein were stored by the corn and ash pig and 1.87 pounds by the corn and protein-free skim milk pig. In the period between 200 to 300 days the figures show a loss of protein. In this time there was a loss in live weight. As this loss was probably mostly fat, the figures for protein storage are no doubt low. The only value of these figures is the contrast between the low gains made in the first 400 days of the experiment and those made in the last half.

The increase in protein content in these pigs is shown graphically in figure 20.

FAT STORED

In the calculation of the amount of fat stored in the body it was assumed that the percentage of fat on the basis of live weight was gradually increasing from 20 to 52 and 55 in the largest pig. This assumption is based on the average composition of pigs of similar size. The two pigs, one fed corn and ash the other fed corn and protein-free skim milk, were slaughtered, so the percentage for fat for the last period is that actually found. The pig fed corn alone was not slaughtered, and the figures were assumed. The figures for the last period of 23 days should be omitted for two of the pigs. In the calculation of the thermal value of the fat stored, 4,200 calories per pound of fat were used.

According to these figures the pig fed corn and ash stored only 9 pounds of fat during the first 400 days and the pig fed corn and protein-free skim milk stored 7 pounds. These figures are probably low.

The increase in fat storage by periods is graphically represented in figure 21. This serves to show the very large increase in fat during the later periods.

RELATION BETWEEN VALUES OF FOOD CONSUMED AND NUTRIENTS STORED

The pounds of protein stored divided into the pounds of protein consumed gives a ratio which serves to show, in a measure, the efficiency of the ration for storage of protein. In section III it has been shown that

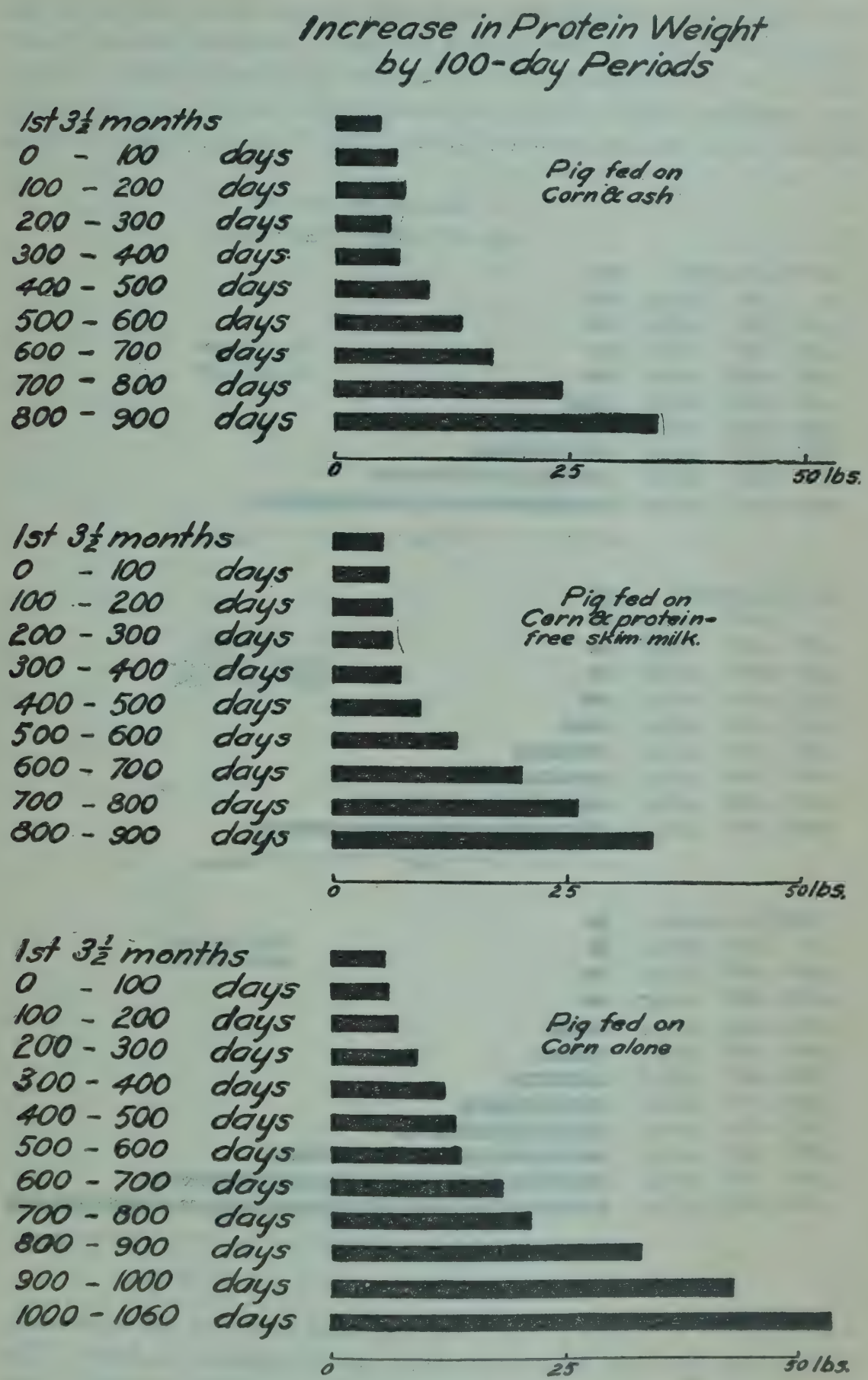

FIG. 20.—Graphical representation of the increase in weight of protein by 100-day periods.

this ratio varies considerably, depending on the ration used. It was shown that with corn alone protein is stored at an average ratio of very nearly 1 to 7. Comparison of the amount of protein stored in the pig fed corn alone and in the pig fed corn and protein-free skim milk with the amount of protein consumed showed that the average ratio for these

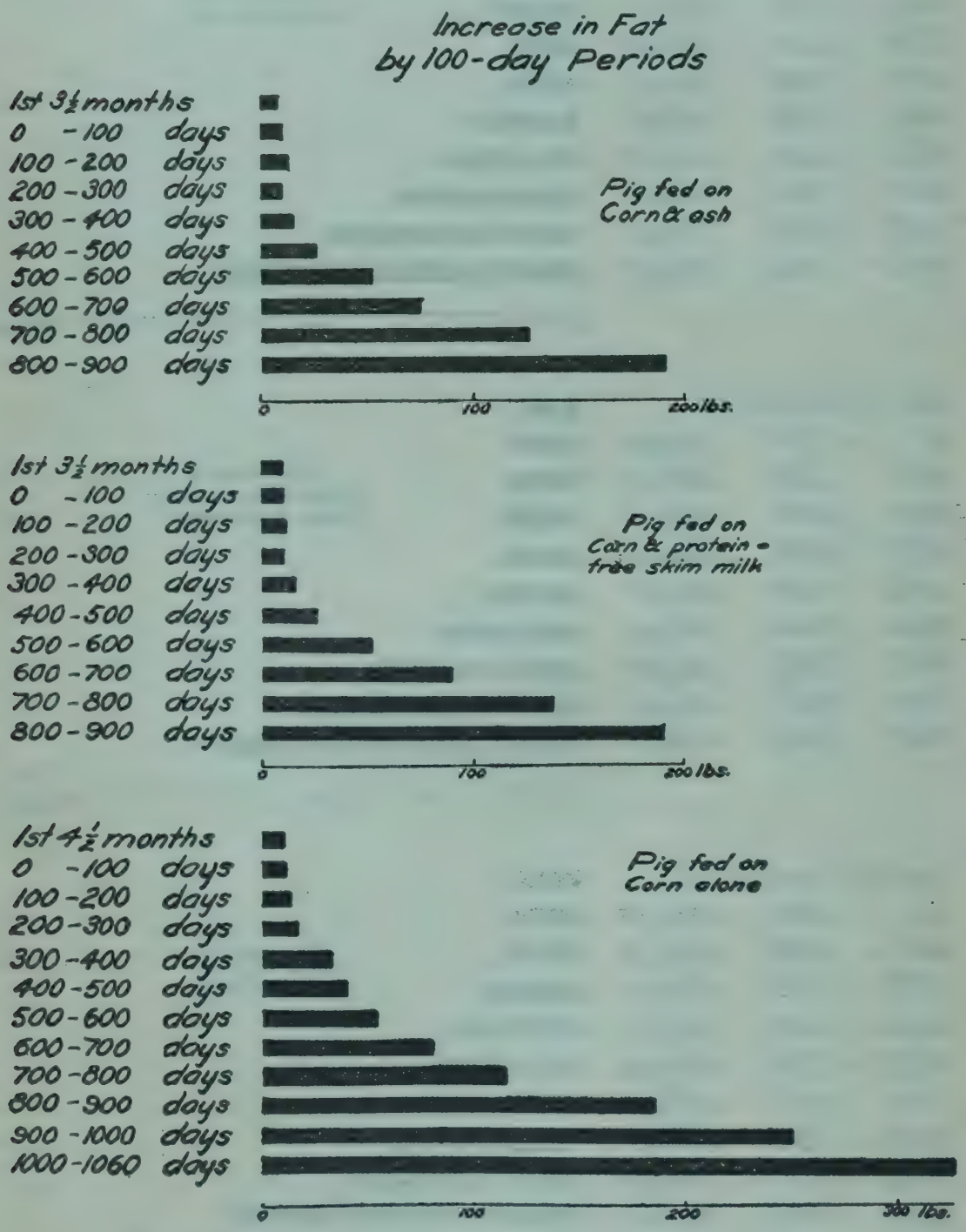

FIG. 21.—Graphical representation of the increase in fat by 100-day periods.

two pigs was very nearly 1 to 7 for the whole period of the experiment. Since these two pigs were slaughtered, this comparison is direct. This means that these two pigs for the whole lifetime stored protein as efficiently as those younger pigs which were fed on corn alone but grew more at an earlier stage. Since all these three pigs did not grow much

the first 400 days of the experiment, it means that the protein was stored at a much wider ratio than 1 to 7 during this period. The detailed figures for the calculations by periods are found in Table XXIV. By dividing the calculated amount of protein stored into the amount consumed, we find that the ratio for the pig fed corn and ash was 1 to 17.61 during the first 400 days and for the pig fed corn and protein-free skim milk, it was 1 to 18.88. And for the pig fed corn alone it was 1 to 12.24 for the first 600 days. This narrow ratio for this pig is due to the fact that this pig had a period of relatively rapid growth between the three hundredth and four hundredth day. During the rest of the life of these pigs they stored protein in a much narrower ratio. The average for the pig fed corn and ash was 5.37 for the last 500 days. For the pig fed corn and protein-free skim milk it was 5.96, and for the pig fed corn alone it was 5.15. While these figures are based on several assumptions founded on experimental evidence, it brings out this fact, that the best ratio in which protein was stored by these pigs fed corn was not far from 1 to 5. While this ratio is narrower than that obtained from pigs fed protein in abundance from other sources than corn, it is much wider than when corn was supplemented by a small amount of protein. The ratio for the pig fed corn and casein, reducing (see section III), was 1 to 3.7, the one fed corn and casein from 1½ pounds of milk for each pound of corn was 1 to 3.3, and that for the one fed corn, starch, casein, and ash was 1 to 2.9.

TABLE XXIV.—*Pounds of fat stored and thermal value*

PIG FED CORN AND ASH

Period.	Weight.	Percentage of fat.	Total fat in body.	Fat stored.	Therms in fat stored.
Days.	*Pounds.*		*Pounds.*	*Pounds.*	
0 to 100 [a]	56	20	11.20	2.80	11.76
100 to 200	65	20	13.00	1.80	7.56
200 to 300	53	20	10.60	−2.40	−10.08
300 to 400	60	25	15.00	4.40	18.48
400 to 500	87	30	26.10	11.10	46.62
500 to 600	131	40	52.40	26.30	110.46
600 to 700	168	45	75.60	23.20	97.44
700 to 800	269	47	126.43	50.83	213.49
800 to 900	383	50	191.50	65.07	273.29
900 to 923	394	52.5	206.85	15.35	64.47
Total				200.85	

TABLE XXIV.—*Pounds of fat stored and thermal value*—Continued

PIG FED CORN AND PROTEIN-FREE SKIM MILK

Period.	Weight.	Percentage of fat.	Total fat in body.	Fat stored.	Therms in fat stored.
Days.	*Pounds.*		*Pounds.*	*Pounds.*	
0 to 100 [b]	51	20	10.20	0.80	3.36
100 to 200	56	20	11.20	1.00	4.20
200 to 300	54	20	10.80	—.40	—1.68
300 to 400	64	25	16.00	5.20	21.84
400 to 500	89	30	26.70	10.70	44.94
500 to 600	132	40	52.80	26.10	109.62
600 to 700	202	45	90.90	38.10	160.02
700 to 800	293	47	137.71	46.81	196.60
800 to 900	379	50	189.50	51.79	217.52
900 to 923	375	52	195.00	5.50	23.10
Total				186.00	

PIG FED CORN ALONE

0 to 100 [c]	58	20	11.60	0.80	3.36
100 to 200	68	20	13.60	2.0	8.40
200 to 300	82	20	16.40	2.80	11.76
300 to 400	128	25	32.00	15.60	65.52
400 to 500	134	30	40.20	8.20	34.44
500 to 600	135	40	54.00	13.80	57.96
600 to 700	180	45	81.00	27.00	113.40
700 to 800	243	47	114.21	33.21	139.48
800 to 900	370	50	185.00	70.79	297.32
900 to 1,000	481	52	250.12	65.12	273.50
1,000 to 1,060	596	55	327.80	77.68	326.26
Total				317.00	

[a] In the 3 1/2 months before the experiment began the pig attained a weight of 42 pounds, of which 20 per cent was fat. The total fat in the body was 8.40 pounds.

[b] In the 3 1/2 months before the experiment began the pig attained a weight of 47 pounds, of which 20 per cent was fat. The total fat in the body was 9.40 pounds.

[c] In the 4 1/2 months before the experiment began the pig attained a weight of 54 pounds, of which 20 per cent was fat. The total fat in the body was 10.80 pounds.

RELATION BETWEEN TOTAL THERMS CONSUMED AND TOTAL THERMS STORED

The pig fed corn and ash consumed 19.31 therms for one stored during the first 400 days of the experiment. During the last 500 days of the experiment he consumed 3.28 therms for one stored. The pig fed corn and protein-free skim milk consumed 18.33 therms for one stored during 400 days of the experiment and 3.11 for one stored during the last 500 days of the experiment. For the pig fed corn alone the therms consumed were 8.23 for the first 600 days and 2.93 for the last 400 days for each therm stored. (Table XXV.)

In section III it was shown that pigs fed corn alone stored on the average 1 therm for 4 consumed. This was with pigs which made some growth on corn alone. In pigs that made a small growth or none at all, the ratio

was much wider. The two pigs compare, in the first 400 days, with those that made a very small growth on corn alone. The pig fed corn alone stored energy a little more efficiently the first 600 days. During the last period of the experiments these pigs compare with the pig fed corn, starch, casein, and ash and the pig fed corn and casein from 3 pounds of milk every seventh day. But when the energy storage is compared with that of the pigs fed corn and medium amounts of milk protein, the ratio is wider. This means that even in the last stages of growth when the increase in live weight was rapid and the gain in proportion to the amount of corn consumed was satisfactory, the energy storage was not as good as with the pigs fed a moderate amount of protein from sources other than corn.

The graphic presentation of the relation between therms consumed and therms stored are given in figure 22.

TABLE XXV.—*Summary of pounds of protein consumed and pounds of protein stored, therms in nutrients consumed and therms in nutrients stored, and ratios*

PIG FED CORN AND ASH

Period.	Protein consumed.	Protein stored.	Ratio.	Average ratio.	Therms in nutrients consumed.	Therms in nutrients stored.	Ratio.	Average ratio.
Days.	*Pounds.*	*Pounds.*						
0 to 100[a]	11.72	1.54	.....		203.89	14.62	13.94	
100 to 200	6.73	.99	.....	17.61	116.96	9.40	12.33	19.31
200 to 300	6.99	— 1.32	.....		121.49	—12.54	Loss.	
300 to 400	9.43	.77	.....		163.93	19.91	8.23	
400 to 500	13.84	2.97	4.66		240.69	52.14	4.61	
500 to 600	22.77	3.53	6.45		396.00	117.03	3.38	
600 to 700	22.00	3.70	5.95	5.37	382.56	104.32	3.67	3.28
700 to 800	37.04	7.41	5.00		643.99	227.64	2.83	
800 to 900	54.10	10.26	5.27		940.67	292.00	3.22	

PIG FED CORN AND PROTEIN-FREE SKIM MILK

0 to 100[a]	7.86	0.44	.....		136.70	4.17	32.78	
100 to 200	7.56	.55	.....	18.88	131.42	5.22	25.17	18.33
200 to 300	7.91	— .22	.....		137.61	— 2.09	Loss.	
300 to 400	10.09	1.10	.....		175.49	23.89	7.34	
400 to 500	14.38	2.75	5.23		250.08	50.06	4.99	
500 to 600	21.67	3.41	6.36		376.85	115.96	3.25	
600 to 700	21.23	7.00	3.03	5.96	369.15	173.04	2.13	3.11
700 to 800	39.47	6.17	6.40		686.29	208.08	3.30	
800 to 900	41.08	3.79	10.84		714.22	224.57	3.18	

PIG FED CORN ALONE

0 to 100[b]	9.07	0.44	.....		157.70	4.18	37.72	
100 to 200	10.75	1.10	.....		187.04	10.45	17.90	
200 to 300	11.68	1.54	.....		203.05	14.62	13.89	
300 to 400	19.44	3.78	.....	12.24	337.94	72.55	4.66	8.23
400 to 500	23.07	.60	.....		401.23	35.56	11.28	
500 to 600	18.53	.10	.....		322.18	58.15	5.54	
600 to 700	19.30	4.50	4.29		335.60	121.77	2.76	
700 to 800	31.84	3.87	8.22		553.59	146.68	3.77	
800 to 900	52.96	11.43	4.63	5.15	920.86	318.58	2.89	2.93
900 to 1,000	65.23	9.99	6.53		1,134.31	292.08	3.88	
1,000 to 1,060	37.25	10.35	3.60		647.71	345.51	1.87	

[a] No data for 3½ months preceding experiment.
[b] No data for 4½ months preceding experiment.

CONCLUSIONS

1. Two pigs, one put on a ration of corn and ash, one on a ration of corn and protein-free skim milk when 3½ months old, did not make satisfactory gains until they had been on this ration for about 500 days.

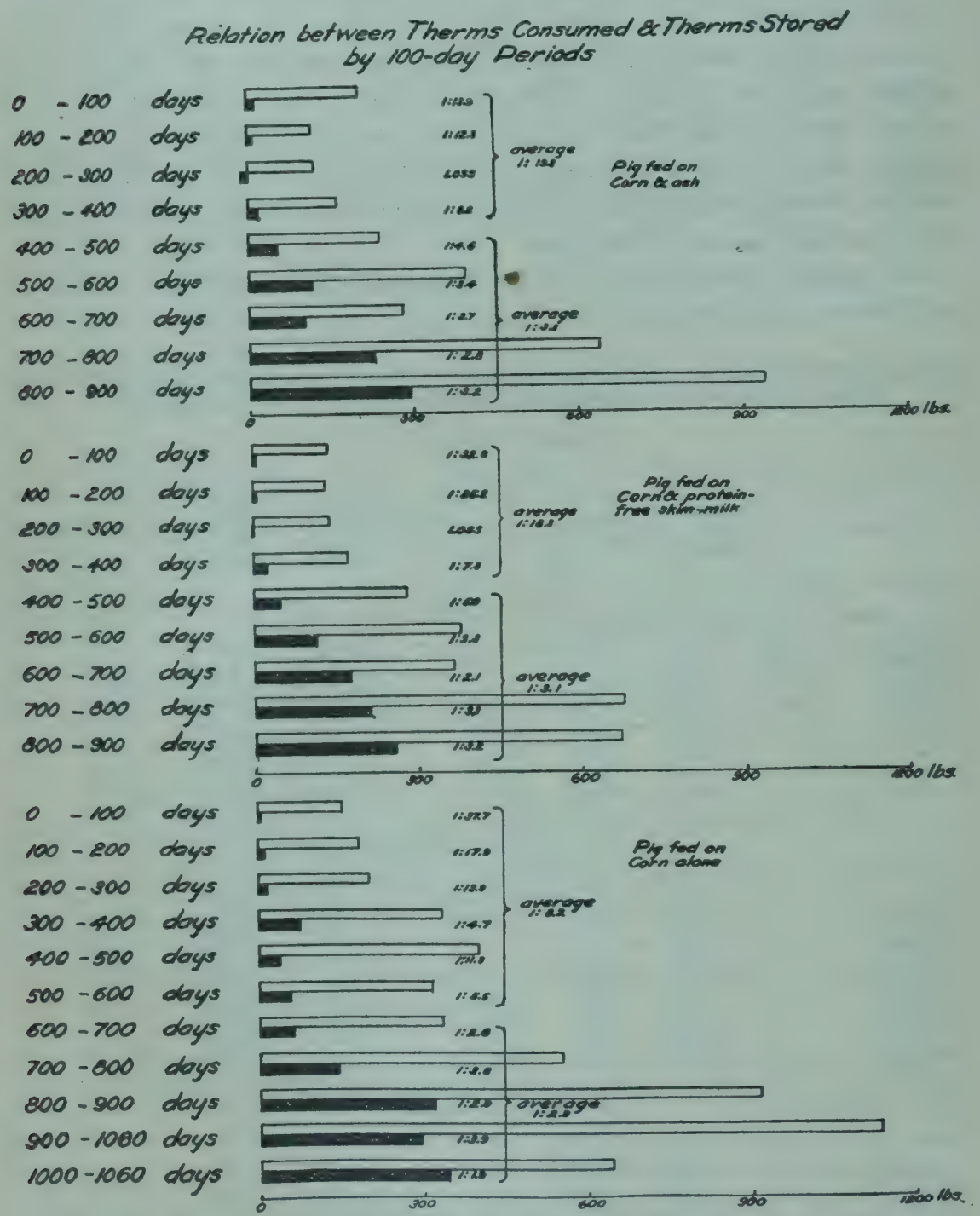

FIG. 22.—Graphical representation of the relation between therms consumed and therms stored by 100-day periods.

From that time for 400 days they made good gains. One other pig put on a ration of corn alone when 4½ months old made very slow gains for about 300 days, more rapid gains the following 400 days, and for the last 360 days of the experiment he made rapid gains.

2. When corn was the sole source of protein in the ration, the ratio of protein stored to protein consumed was 1 to 7. This ratio was obtained when corn was fed for a prolonged period—900 days. This agrees with the ratio obtained in several other instances when corn was fed for a period of 180 days. This appears to be the limit of the possibilities of protein storage from corn.

3. The two pigs, one fed corn and ash, the other fed corn and protien-free skim milk, stored 1 therm for 18.82 consumed during the first 400 days of the experiment. During the last 500 days they stored 1 therm for 3.19 consumed. The pig fed corn alone stored 1 therm for 8.23 consumed during the first 600 days. During the last 400 days he stored 1 therm for 2.93 consumed.

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SOME FUNDAMENTALS OF STABLE VENTILATION

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COOPERATIVE INVESTIGATIONS BETWEEN THE BUREAU OF ANIMAL INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE AND THE INSTITUTE OF ANIMAL NUTRITION OF THE PENNSYLVANIA STATE COLLEGE

Much study has been given in the past to the mechanical aspects of stable ventilation and to the construction of efficient systems; but, so far as the authors are aware, singularly little attention has been paid to the amount of motive power available for the operation of these systems. This is particularly true of that portion of it which is derived from the heat production of the animals, notwithstanding the fact that the latter may vary within wide limits and may at times constitute the sole motive power.

In the cooperative investigations upon animal nutrition which have been carried on at this institute during the past 20 years, a large number of direct determinations of the heat production of cattle under different conditions have been made. Numerous similar determinations have also been made by Kellner (*11*, *12*, *13*)[1] by the method of indirect calorimetry. A smaller number of results upon other species of farm animals are also on record. Upon the initiative and with the efficient cooperation of Mr. W. B. Clarkson, chairman of the Committee on Farm Building Ventilation of the American Society of Agricultural Engineers, we have attempted to work out a method by which these results may be applied to the problems of stable ventilation and the heat production in any specific case computed with a fair approximation to accuracy. The present paper contains the results of these endeavors.

INTRODUCTION

The installation of an effective ventilating system must necessarily depend, first, on a knowledge of the scientific principles involved and, second, upon a study of the conditions of each individual case. As the amounts of pure air required by the different species of farm animals are different, the minimum volume of air movement through a stable must vary accordingly. The construction of a ventilating system must be based on the unit of air movement chosen and on the available motive power which is to ensure the required supply of air.

[1] Reference is made by number (italic) to "Literature cited," p. 367–368

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The motive power utilized in stable ventilation is chiefly the passing wind and the heat and water vapor given off by the animals. It frequently happens, however, that the motive force due to the wind is very small or even zero. At such times the air movement is entirely dependent upon the motive power derived from a rise in temperature and from an increase in the moisture content of the air after it enters the stable. It is therefore important to know this minimum motive power—that is, the heat and water vapor supplied by the animals, as this knowledge is evidently fundamental in determining the proper dimensions of the ventilating system.

But the heat given off by farm animals, while serving as a motive power for ventilation, is also relied upon to maintain the temperature of the stable at a comfortable degree in cold weather. The Committee on Farm Building Ventilation of the American Society of Agricultural Engineers in its recent report (*8*) to the society has emphasized very strongly the need of effective control of the temperature in farm buildings, as based on a number of its investigations. The committee found many barns with well-equipped ventilating systems which were not producing satisfactory results to the owners, not because of inadequate ventilation, but because of the fact that the buildings were too cold. All these facts point to the necessity of knowing how much heat is given off by the different farm animals and is available for heating and ventilating purposes.

Besides heat and water vapor farm animals give off carbon dioxid and some volatile organic products. Air once respired contains carbon dioxid in a quantity which makes it unfit to be breathed again unless very much diluted with pure air. It is chiefly the carbon-dioxid content of the air that serves as a basis for determining its degree of purity. In selecting a unit of air movement in the construction of a ventilating system it appears, therefore, that a knowledge of the average amounts of carbon dioxid produced by the different animals must be of not a little significance.

AMOUNT OF AIR REQUIRED FOR DIFFERENT SPECIES AND STANDARD OF PURITY

From what has just been said it is clear that the question of stable ventilation is a question of maintaining the proper purity of air in the stable as well as the proper temperature. Air supports the life of the animal. The air an animal breathes is as much an indispensable part of the feed it consumes as is the hay or the grain eaten. Within the animal body neither assimilation of food nor generation of energy can take place without the consumption of a proportionate amount of air. When the animals are outside they have plenty of pure air at their disposal; in the stable, however, the air is contaminated with the gases thrown off by them. Unless there is an air movement at a proper rate into and

out of the stable, these gases—the carbon dioxid and the volatile organic products exhaled by the animals—will accumulate and may have injurious effects on them.

King (*14*) bases his estimates of the volume of air which should move continuously through stables, first, on the amount of pure air which must be breathed by different animals and, second, on the standard of purity recommended by him. According to his computations a horse must draw into and force out of his lungs, on the average, each hour, some 142 cubic feet of air, the cow 117, the pig 46, and the sheep 30 cubic feet.

The standard recommended by King requires a degree of purity of air not lower than 96.7 per cent.—that is, that the air in the stable shall at no time contain more than 3.3 per cent of air once breathed. Since the air coming from the lungs contains about 4.24 volume per cent of carbon dioxid and pure air 0.028 volume per cent, it appears that King's standard allows $4.24 \times 0.033 + 0.028 \times 0.967$, or 0.167 volume per cent of carbon dioxid. From the amounts of air breathed by the different animals given above, the rate at which air must enter and leave the stable to correspond to this standard is computed per hour and per head as follows:

$$\text{For horses: } \frac{142 \times 100}{3.3} = 4{,}303 \text{ cubic feet.}$$

$$\text{For cows: } \frac{117 \times 100}{3.3} = 3{,}545 \text{ cubic feet.}$$

$$\text{For swine: } \frac{46 \times 100}{3.3} = 1{,}394 \text{ cubic feet.}$$

$$\text{For sheep: } \frac{30 \times 100}{3.3} = 909 \text{ cubic feet.}$$

It is not claimed that the standard of air purity and the units of air movement for the different animals given above are absolutely needed. While they probably afford a good gauge by which to be guided, they have been given here mainly to illustrate the method and the basis of their computation.

MOTIVE POWER FOR STABLE VENTILATION

The maintenance of a flow of air through a building requires the continuous expenditure of energy, and the amount of this energy and the work done will be in direct proportion to the weight of air moved through the ventilated space and the resistance it is necessary to overcome in accomplishing this movement. To supply air to 100 horses, for example, at the rate of 4,303 cubic feet per hour and per head, the necessary amount of work is that of moving through the stable each hour $\frac{4{,}303 \times 0.08 \times 100}{2{,}000}$ or 17.2 tons, 0.08 representing the weight of 1 cubic foot of air in pounds.

The power used to accomplish the air movement through stables, as already stated, is chiefly the passing wind and the heat and moisture given off by the animals. The motive force due to the wind depends on its velocity, direction, etc., and is, in general, very variable and sometimes

even zero. On the other hand, the amounts of heat and water vapor given off by animals are fairly constant under like conditions and must, therefore, be depended upon to cause a flow of air sufficient to supply the minimum amount needed. Since it is this minimum air movement that concerns us most, only the motive power derived from the heat and water vapor produced by the animals will be considered here.

ANIMAL HEAT AS A MOTIVE POWER IN VENTILATION

The immediate cause of air movement into and out of a ventilated space is a difference of pressure established between the air in the space to be ventilated and that outside. The effect of the heat given off by the animals is to render the air of the stable relatively lighter than the air outside. This difference in density causes a difference in pressure, which tends to maintain a continuous flow of air into and out of the stable.

The difference in pressure between the air in the barn and that outside resulting from a difference in temperature can be computed in the following way: When air is warmed its volume expands 1/491 of its volume at 32° F. for each degree Fahrenheit rise in temperature. This expansion tends to force out 1 cubic foot of air for each 491 cubic feet contained in the stable, and the air remaining will consequently weigh less than an equal volume outside by an amount equal to the weight of the air thus forced out. If, for example, in a stable containing 19,640 cubic feet, or one very nearly 40 by 40 by 12.3 feet, the temperature is raised to 57°, as compared with 32° outside, the air forced out by expansion will be $\frac{19,640 \times 25}{491}$, or 1,000 cubic feet. In other words, the air remaining will weigh 80 pounds less than an equal volume outside. This means that the total pressure into the stable at the floor is 80 pounds greater than that exerted outward by the inside air; and, since the floor has an area of $40 \times 40 = 1,600$ square feet, the pressure tending to force air into the stable at a floor opening and out at the ceiling is $\frac{80}{1,600}$, or 0.05 pound per square foot.

Based on the foregoing considerations, the magnitude of the temperature effect in producing draft, according to King, is represented by the following equation:

$$\text{Cubic feet per hour} = 60 \times 60 \times 8\sqrt{\frac{T-t}{491}}H,$$

in which—

60×60 is the number of seconds per hour,
8 is $\sqrt{2g}$, g being the value of gravity, 32.16 feet per second,
T is the temperature of air inside,
t is the temperature of air outside,
H is the height of room or ventilator,
1/491 is the expansion of air for 1° F.

This equation gives the theoretical value of the air flow per square foot of cross section. The actual flow of air is, however, less than the theoretical, its relative value depending on the resistance which the moving air has to overcome. It appears from an examination of the equation that in order to determine the flow of air the height and the cross section of the ventilator and the difference in temperature between the inside and outside air must be known. On the other hand, from the unit of air movement chosen and the difference in temperature likely to exist the minimum size of the ventilator can be determined.

What difference in temperature can be maintained by the heat given off by the different animals when the ventilation is sufficient to supply the minimum amount of air needed is a question which will be considered later in its relation to heat emission.

WATER VAPOR AS A MOTIVE POWER IN VENTILATION

The water vapor which the animals give off is under ordinary conditions taken up by the moving air and carried off as rapidly as it is formed. The addition of the water vapor to the air in the stable tends to make it lighter than that outside, and this effect serves as a measure of the influence of water vapor as a motive power in ventilation.

The higher the temperature of the air the greater is its moisture-holding capacity. The more moisture the air contains the lighter it is. Consequently, the motive power derived from the water vapor is greater the higher the temperature of the stable air and the more completely it is saturated.

The quantities of water vapor produced by the different animals are given later. The motive power derived from the moisture is much less than that derived from the heat; and, since its magnitude largely depends on the difference in temperature, it can be considered only as secondary in importance.

PRINCIPLES INVOLVED IN THE PRODUCTION OF HEAT BY ANIMALS

The function of the animal heat as a motive power for stable ventilation has already been considered. A second function of the animal heat is to keep the stable warm in cold weather. The optimum temperature to be maintained in the stable varies with the kind of animal and also with the ration. There exists also a certain relation between the heat given off by the animal and its thermal surroundings. For the purpose of estimating approximately the best stable temperature, both as regards comfort and economy, it appears desirable to turn to a consideration of the scientific principles involved.

REGULATION OF BODY TEMPERATURE

Farm animals belong to that general class known as warm-blooded animals, whose bodies during health maintain a nearly constant temperature which is the resultant of two factors, thermogenesis, or the

development of heat inside the body, and thermolysis, the loss of heat from the body, principally by radiation and conduction and as latent heat of water vapor. The external temperature tends to influence the outflow of heat, but the animal is able to regulate it by physical and chemical methods.

There is a certain external temperature, called the critical temperature, at which the outflow of heat just balances the necessary heat production of the animal as a result of internal work. Above this temperature the radiating capacity of the body surface is varied to meet the varying conditions; below it, this method of regulation is largely exhausted, and therefore more or less oxidation of tissue is required to maintain the normal temperature of the body.

EFFECT OF FEED CONSUMPTION ON THE HEAT PRODUCTION AND ON THE CRITICAL TEMPERATURE

It is a fact demonstrated by numerous experiments that the consumption of feed results in increasing the heat production of an animal. When an animal is fasting it produces a certain amount of heat due to the vital functions of the internal organs. This is generally termed basal, or fasting katabolism. When the animal is fed, its heat production is increased over that of the fasting state. This increment of heat brought about by the consumption of feed has been ascribed to various causes, one of which is the expenditure of energy in the digestion and assimilation of the feed, often collectively termed "work of digestion."

The more heat the animal produces the more cold it naturally can withstand without being compelled to oxidize body tissue in order to maintain the normal body temperature. This, in other words, means that the consumption of feed lowers the critical temperature, the effect varying with the nature and quantity of the feed. Animals fed heavily, as in productive feeding, can therefore withstand more cold, or have a lower critical temperature, than animals kept on a simple maintenance ration, while the critical temperature of the latter is higher than that of the fasting animal.

EFFECT OF LOW THERMAL SURROUNDINGS ON MAINTENANCE REQUIREMENT

From what has just been said it appears that a ration sufficient for maintenance at a temperature higher than the critical may be insufficient for maintenance when the thermal surroundings are lower than the critical, because of the failure to meet the demand for heat. What the critical temperatures of the different farm animals are is therefore a question not only of physiological but also of economic significance.

It is apparent, however, that the critical temperatures of farm animals do not lend themselves to accurate determination. At best they are estimated only approximately. A summary of the results of different

investigators on this subject (*3, p. 312*) shows that the critical external temperature for the horse is high as compared with that of ruminants, so that a ration which is sufficient for maintenance in summer may be insufficient in winter. The critical temperature for swine has been likewise found to be comparatively high (68° to 73° F.), which means that exposure to low temperatures may be expected to increase the actual maintenance ration and the heat production of swine, and this has been confirmed by experimental results. On the other hand, the results on cattle seem to indicate that their critical temperature is rather low (much below 56°), which means that cattle can be exposed to lower temperatures than horses or swine before their maintenance requirement will be affected and their heat production stimulated.

EFFECT OF LOW THERMAL SURROUNDINGS ON PRODUCTIVE FEEDING

The production of meat or milk implies the consumption of large quantities of feed. Since the latter is the source of a large amount of heat, due to the "work of digestion," which has to be removed at a correspondingly rapid rate, it appears that heavy producers are better adapted to relatively cold thermal surroundings. Furthermore, since it is the aim in feeding such animals to induce them to eat as much feed as can be economically converted into useful products, it seems desirable, on the one hand, that the thermal surroundings should be low in order to maintain the appetite of the animals and, on the other, not so low as would cause wasteful oxidation for simple heat production.

The question whether winter feeding for fattening can be accomplished to better advantage in the stable than in the open shed has interested many investigators, and a considerable amount of experimental work is on record. The results (1) show in general that cattle are best adapted to exposure—that is, they produce as good results when exposed as when stable fed. Swine are least adapted to exposure, the gain of the animals exposed to severe weather being frequently negative, while sheep seem to take an intermediate place. These results are in full harmony with the findings given above as regards the critical temperature. Less decisive results have been obtained with dairy cows, but it appears fairly well established that for well-fed animals the need for warm stables has been somewhat overemphasized.

OPTIMUM STABLE TEMPERATURE

Both theoretical considerations and the results of experience show that a certain excess of heat production over that absolutely required to maintain the body temperature is likely to be advantageous, both by promoting the comfort of the animal and as providing a margin of safety. On the other hand, an unnecessarily high temperature tends to affect the appetite and general health of the animal. From this it follows that the best thermal surroundings for animals lie between these limits—

namely, somewhat above the critical point, but not so much as to affect the appetite and thrift. These limits, evidently, will vary with the species of animal and with the amount and character of the ration. The best temperature surroundings for animals being fed high, according to King, are likely to lie between 45° and 50° F., while for animals on a maintenance ration, the best temperatures may be between 55° and 65°. For dairy cows having large udders only scantily clothed with hair and through which much blood must flow, a temperature as high as 50° to 60° is considered as probably the best.

Whether the heat eliminated by animals is sufficient to maintain in the stable approximately these temperatures in cold weather when the air movement is at the proper rate will be considered on subsequent pages.

METHOD OF COMPUTING HEAT PRODUCTION

The discussions of the foregoing paragraphs make it evident that the heat produced by an animal may be regarded as the sum of two factors, first the necessary internal work due to the vital activities of the internal organs and, second, the "work of digestion." The first gives rise to an amount of heat equivalent to the fasting katabolism, which varies with the species and size of the animal, while the second gives rise to an increment of heat due to feed consumption, which varies with the character and quantity of the ration. It is clear, then, that no single standard value can be assumed as representing even approximately the heat production of any species.

When the fasting katabolism of an animal and the heat increment due to the feed eaten are known, it is evident that the total heat produced by the animal can be computed by simple addition. Experimental data are available from which it is possible to estimate more or less accurately the fasting katabolism of farm animals according to their live weights, and also the increment of heat due to the ration feed. These data have been used as the general basis for computing the heat production. The details of the method are best illustrated by the computation on subsequent pages of the heat production of dairy cows.

ACCURACY OF THE COMPUTATIONS

The reader should beware of being led by the apparently very exact figures of the tables on succeeding pages to ascribe to these data a greater degree of accuracy than they really possess.

In the first place they represent specific cases assumed to be more or less typical. The actual production of heat, carbon dioxid, and water by a given species will show wide variations from stable to stable, and in the same stable from time to time, according to the size of the animals, their degree of activity, and especially the amounts of feed which they consume.

In the second place, it is by no means intended to assert on page 354, for example, that every Jersey cow weighing 750 pounds and yielding 20 pounds of 5 per cent milk will produce exactly 16,313 calories of total heat. The computations have been made on the basis of average results from which those on an individual animal may vary considerably. This is especially true of the fasting katabolism. Moreover, the computations have been carried out to the nearest whole calorie so as to record the exact results of the calculation. In view, however, of the many possibilities of experimental error involved, it seems very questionable whether the last three digits are significant. Probably an estimate to the nearest thousand calories—that is, to one therm—would be all that is justified and would be sufficiently accurate for the discussion of all ventilation problems.

HEAT PRODUCTION OF CATTLE

FASTING KATABOLISM

The data for the fasting katabolism of cattle, although obtained by indirect methods and not by actually starving the animals, are more trustworthy than those for any other species of farm animals for the reason that they are more abundant, are concordant, and are based largely on experiments with the respiration calorimeter by means of which direct determinations of the heat production were made. In these experiments the animal is usually fed two different amounts of the same feed, and the effect of this on the heat production—that is, the decrease of heat production per pound decrease of feed—is determined. From this it is estimated how much heat would be produced if all the feed were withdrawn—that is, if the animal were reduced to the fasting state.

The average given by Armsby (*3, p. 711*) for the fasting katabolism of cattle per 1,000 pounds live weight is 6 therms, or 6,000 calories, and, as the fasting katabolism of animals of the same species has been found to be approximately proportional to the two-thirds power of their live weight, that of cattle is computed accordingly. Table I gives the fasting katabolism of cattle according to their live weight in terms of calories.

TABLE I.—*Fasting katabolism of cattle*

Live weight.	Calories, per head.	Live weight.	Calories, per head.
150	1,690	1,000	6,000
250	2,380	1,250	6,960
500	3,780	1,500	7,860
750	4,950		

HEAT INCREMENT DUE TO THE CONSUMPTION OF FEED BY CATTLE

The energy expended by cattle in the increased body activities connected with the digestion and assimilation of many feeding stuffs have been determined directly by varying the amount of the feeding stuff in

the ration and by determining the heat production. From a comparison of two determinations the heat increment caused by a pound of feeding stuff can be determined. The data for the heat increment caused by different feeding stuffs are average figures computed by Armsby and Fries (*4, 5*) from the results of their own experiments and those of Kellner and Köhler (*11, 12, 13*) on beef cattle and are given in Table II in calories per pound of dry matter. The corresponding figures for dairy cows would probably be somewhat less, but how much less has not yet been determined.

TABLE II.—*Increment of heat production by cattle per pound of dry matter consumed*

Feeding stuffs.	Experimenters.	Energy expenditure.
		Calories.
Roughage:		
Timothy hay	Armsby and Fries	354.7
Red clover hay	do	441.3
Do	Kellner and Köhler	422.7
Mixed hay	Armsby and Fries	444.5
Alfalfa hay	do	530.3
"Grass hay"	Kellner and Köhler	474.0
Meadow hay	do	568.8
Rowen	do	434.6
Corn stover	Armsby and Fries	483.1
Barley straw	Kellner and Köhler	397.8
Oat straw	do	460.0
Wheat straw	do	516.2
Straw pulp	do	526.2
Concentrates:		
Corn meal	Armsby and Fries	583.3
Hominy chop	do	619.2
Wheat bran	do	533.9
Cottonseed meal	Kellner and Köhler	443.6
Linseed meal	do	547.9
Palmnut meal	do	456.8
Peanut meal	do	525.7
Beet molasses	do	448.2
Starch	do	566.1
Peanut oil	do	783.4
Wheat gluten	do	950.8

In its relations to stable ventilation the computation of the heat production of cattle is of special interest in the case of dairy cows, since in cold climates these animals are almost always stabled during the winter while beef cattle are quite commonly fed in the open. The computations for cows are therefore given in considerable detail in the following paragraphs.

TYPICAL WEIGHTS AND RATIONS OF DAIRY COWS

Obviously, no single value can be given for the heat production of the dairy cow, since it varies widely according to the size of the animal and the amount of feed consumed. All that is possible is to select certain typical live weights and rations and to compute the corresponding heat production as illustrations of the method in its application to specific

cases, such as may be found in good practice. The typical weights and rations upon which the computation of the heat emission by dairy cows has been based are those suggested, at the request of Mr. Clarkson, by Prof. C. H. Eckles, of the University of Minnesota.

TABLE III.—*Typical live weights and rations*

Breed.	Live weight.	Daily milk yield.		Daily ration.			
		Pounds.	Percentage of fat.	Corn silage.	Alfalfa hay.	Grain mixture.[a]	Additional linseed meal.
	Pounds.			*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Jersey	900	20	5.0	30	8	6	.....
Do	900	30	5.0	30	8	9	1
Holstein	1,250	30	3.5	40	10	6	.....
Do	1,250	45	3.5	40	10	10	1

[a] Composed of ground corn 4 parts, wheat bran 2 parts, linseed meal 1 part.

COMPUTATION OF THE HEAT PRODUCTION OF DAIRY COWS

To compute, for example, the daily heat production by the typical Jersey cow giving 20 pounds of milk daily, it is only necessary to add to the fasting katabolism of a cow weighing 900 pounds the heat increment due to the ration consumed. According to Table I the fasting katabolism of a cow weighing 1,000 pounds is 6,000 calories, while that for one weighing 750 pounds is 4,950 calories. From these figures the fasting katabolism of a cow weighing 900 pounds may be estimated with sufficient accuracy by simple proportion as being 5,580 calories per day.

It remains to figure out the heat increment caused by the consumption of feed. By using the average percentages of dry matter in the feeds as given in Henry and Morrison's tables (*10*) it is found that the amounts of dry matter contained in the ration are:

In corn silage..... 7.9 pounds.
In alfalfa hay..... 7.3 pounds.
In grain mixture..... 5.4 pounds.

Since the grain mixture consists of 4 parts of corn, 2 parts of wheat bran, and 1 part of linseed meal, the heat increment per pound of dry matter of the grain mixture (Table II) is:

$$1/7(583.3\times4+533.9\times2+547.9\times1)=564.1 \text{ calories.}$$

The heat increment per pound of dry matter of alfalfa hay is 530.3 calories, and that of corn silage is assumed to be 483.1 calories—that is, the figure corresponding to corn stover—since no figure for corn silage is available. From these figures the total heat increment caused by the ration is obtained as follows:

Corn silage.....483.1×7.9= 3,816 calories.
Alfalfa hay.....530.3×7.3= 3,871 calories.
Grain mixture.....564.1×5.4= 3,046 calories.

Total heat increment.....10,733 calories.

Adding to this the fasting katabolism of the Jersey cow, 5,580 calories, gives the total heat production per day as 16,313 calories.

Computed by this method, the total heat production by the cows per day per head is shown in Table IV.

TABLE IV.—*Total heat production by typical cows per day per head*

Animal.	Calories.
Jersey cow producing 20 pounds of milk	16,313
Jersey cow producing 30 pounds of milk	18,273
Holstein cow producing 30 pounds of milk	19,905
Holstein cow producing 45 pounds of milk	22,372

HEAT GIVEN OFF BY RADIATION AND CONDUCTION AND AS LATENT HEAT OF WATER VAPOR

The heat emission as computed above includes that given off by radiation and conduction and the latent heat of the water vaporized. The latent heat of water vapor, however, can hardly be regarded as available for ventilation purposes, inasmuch as under average conditions there is no considerable accumulation of condensed water, the water vapor being removed from the barn as rapidly as it is produced by the animals so that there is little chance for its latent heat to be liberated.

From the experiments on cattle with the respiration calorimeter it has been found that except on very heavy rations the latent heat of water vapor constitutes approximately 25 per cent of the total heat given off, while about 75 per cent is eliminated by radiation and conduction. On this basis, the heat emission by radiation and conduction and as latent heat of water vapor per day per head for cows has been computed (Table V).

TABLE V.—*Heat given off by radiation and conduction and as latent heat of water vapor per day per head by typical cows*

Animal.	Heat emission by radiation and conduction.	Latent heat of water vapor.
	Calories.	*Calories.*
Jersey cow producing 20 pounds of milk	12,235	4,078
Jersey cow producing 30 pounds of milk	13,705	4,568
Holstein cow producing 30 pounds of milk	14,929	4,976
Holstein cow producing 45 pounds of milk	16,779	5,593

WATER VAPOR PRODUCED BY COWS

The computation of the amount of water vapor produced by an animal when the latent heat of the water vapor is known is a very simple matter. The latent heat of 1 gm. of water vapor is 0.587 calorie. By

dividing, therefore, the calories of latent heat of water vapor by 0.587 the number of grams of water vapor produced is obtained. In the foregoing cases the computed amounts per day and per head are as shown in Table VI.

TABLE VI.—*Water vapor produced by typical cows per day and per head*

Animal.	Water vapor.
	Gm.
Jersey cow producing 20 pounds of milk	6,947
Jersey cow producing 30 pounds of milk	7,782
Holstein cow producing 30 pounds of milk	8,477
Holstein cow producing 45 pounds of milk	9,528

CARBON DIOXID PRODUCED BY COWS

Since carbon dioxid is a product of combustion, however slow it may be, within the animal body, it is natural to expect that a more or less definite relation between the heat production and the output of carbon dioxid must exist. From the accumulated data of the heat emission and carbon dioxid production by cattle determined directly by means of the respiration calorimeter at this Institute, the relation of the carbon dioxid produced to the heat given off has been very recently established by Armsby, Fries, and Braman (*6*), who, on comparing the daily output of carbon dioxid and the heat production by steers and cows for 188 separate days, found that in each case the ratio of the carbon dioxid produced in grams to the total heat emission in calories was very close to 1 to 2.5, or 0.4, the mean ratio being 1 to 2.495. By making use of this factor the amount of carbon dioxid produced by cows in grams is computed by simply multiplying the calories of daily heat emission by 0.4.

TABLE VII.—*Carbon dioxid produced by cows per day and per head*

Animal.	Carbon dioxid.
	Gm.
Jersey cow producing 20 pounds of milk	6,525
Jersey cow producing 30 pounds of milk	7,309
Holstein cow producing 30 pounds of milk	7,962
Holstein cow producing 45 pounds of milk	8,949

HEAT PRODUCTION OF COWS ON MAINTENANCE

The daily maintenance ration of cattle has been computed by Armsby (*2*) from a number of experiments in terms of metabolizable energy, which in this case also represents the total heat production, 10,500 calories being the average per 1,000 pounds live weight. This, com-

puted in proportion to the two-thirds power of the live weight, gives 9,788 and 12,184 calories, respectively, as the daily total heat production on maintenance by the Jersey and Holstein cows of the assumed weights. The heat emission by radiation and conduction, the water vapor, and the carbon dioxid produced by cows on maintenance have been computed by the methods just described and are included in the summary of the results of the computations on dairy cows in Table VIII.

TABLE VIII.—*Heat, water vapor, and carbon dioxid produced by typical cows per day and per head*

Animal.	Total heat emission.	Heat emission by radiation and conduction.	Latent heat of water vapor.	Water vapor.	Carbon dioxid.
	Calories.	*Calories.*	*Calories.*	*Gm.*	*Gm.*
Jersey cow producing 20 pounds of milk	16,313	12,235	4,078	6,947	6,525
Jersey cow producing 30 pounds of milk	18,273	13,705	4,568	7,782	7,309
Holstein cow producing 30 pounds of milk	19,905	14,929	4,976	8,477	7,962
Holstein cow producing 45 pounds of milk	22,372	16,779	5,593	9,528	8,949
Jersey cow on maintenance	9,788	7,341	2,447	4,169	3,915
Holstein cow on maintenance	12,184	9,138	3,046	5,189	4,874

HEAT PRODUCTION OF HORSES

HEAT INCREMENT DUE TO THE CONSUMPTION OF FEED

While the general principles of the computation of the fasting katabolism of horses are essentially the same as for cattle, the computation of the heat increment due to the consumption of feed is a much more complicated process. The reason for this lies in the fact that no calorimetric experiments for the direct determination of the heat production and of the balance of energy have been made with horses, and consequently an indirect method of computation of the heat increment has to be resorted to, which involves many estimates and calculations.

From the results of their experiments, Zuntz and Hagemann (*18*) estimate that the metabolizable energy of the feed of a horse equals 1,796 calories per pound of digestible nutrients; and they assume, on the basis of Magnus Levy's experiments on man, that 9 per cent of the metabolizable energy of the digestible nutrients consumed by a horse is converted into heat in the process of digestion. They furthermore compute from the results of their own experiments that each pound of total crude fiber consumed increases the heat production by 1,202 calories additional, so that the total heat increment due to consumption of feed by the horse is the sum of these two amounts. It is obvious that this method of computation necessitates a knowledge of the digestibility and of the total crude fiber content of the feed.

To illustrate the method of computing the heat increment due to the consumption of feed, that caused by a pound of oats is calculated as follows, the digestible nutrients being those given in Bulletin 186 of the Kansas Experiment Station (*15*), with the exception of those for clover hay, which are taken from Henry and Morrison's tables (*10*), from which have also been obtained the data for total crude fiber.

Digestible nutrients of oats

Protein	0.107 pound.
Carbohydrates	0.503 pound.
Fat (3.8×2.4)	0.092 pound.
Total	0.702 pound.
Total crude fiber	0.109 pound.
Metabolizable energy	1,796 calories×0.702=1,260.8 calories.

Heat increment

9 per cent metabolizable energy	1,260.8×0.09 =113.5 calories.
Additional for crude fiber	1,202 ×0.109=131.0 calories.
Total heat increment	244.5 calories.

The heat increment caused by other feeding stuffs has been computed in a similar manner.

FASTING KATABOLISM

The total heat production of the horse was estimated by Zuntz and Hagemann (*18*) from the respiratory exchange determined by means of a respiration apparatus for short periods after the consumption of different rations. From a comparison of the results and by the indirect method of computing the heat increment due to the work of digestion which has been illustrated they have computed the average fasting katabolism of the horse to be 4,100 calories per 1,000 pounds live weight. In proportion to the two-thirds power of the live weight the fasting katabolism of horses, as computed by Armsby, is given in Table IX.

TABLE IX.—*Fasting katabolism of horses*

Live weight.	Fasting katabolism per head.	Live weight.	Fasting katabolism per head.
	Calories.		*Calories.*
150	1,160	1,000	4,100
250	1,630	1,250	4,760
500	2,580	1,500	5,370
750	3,390		

TYPICAL WEIGHTS AND RATIONS OF HORSES

As examples of typical live weights and rations for horses upon which to base the computation of the heat production of these animals the following, suggested, at the request of Mr. Clarkson, by J. L. Edmonds, of the Illinois College of Agriculture, have been used.

Daily ration for light horses that get only about enough exercise to keep them in good condition per 1,000-pound horse:

8 pounds of oats.
2 pounds of wheat bran.
10 pounds of timothy hay.

Daily ration for 1,500-pound geldings at moderate work:

9 pounds of oats.
9 pounds of ear corn.
7½ pounds of timothy hay.
7½ pounds of clover hay.

TOTAL HEAT PRODUCTION

The total heat increment due to the ration fed, computed in the manner described, plus the fasting katabolism of the horse, gives the total heat production of horses of the weights and consuming the rations just specified when performing no work. The heat production is, of course, greatly increased during work, but plainly this additional heat is not ordinarily available as motive power for ventilation.

TABLE X.—*Total heat production per day and per head by typical horses at rest*

Animal.	Live weight.	Heat.
	Pounds.	*Calories.*
Light horses	1,000	10,853
Heavy work horses	1,500	14,835

HEAT EMISSION BY RADIATION AND CONDUCTION, WATER VAPOR, AND CARBON DIOXID PRODUCED BY HORSES

On the assumption that approximately the same relation exists as in the case of cattle between the total heat emission and the latent heat of water vapor and between the heat and the carbon dioxid produced by horses, a computation was made of the heat given off by radiation and conduction, the latent heat of the water vapor, the amount of water vapor, and the amount of carbon dioxid produced by horses. The method described above for cows was used, and the results are shown in Table XI.

TABLE XI.—*Heat emission by radiation and conduction, water vapor, and carbon dioxid produced per day and per head by typical horses at rest*

Animal.	Live weight.	Heat emission by radiation and conduction.	Latent heat of water vapor.	Amount of water vapor.	Carbon dioxid.
	Pounds.	*Calories.*	*Calories.*	*Gm.*	*Gm.*
Light horses	1,000	8,140	2,713	4,622	4,341
Heavy work horses	1,500	11,126	3,709	6,319	5,934

HEAT PRODUCTION OF SWINE

FASTING KATABOLISM

The fasting katabolism of swine has been determined independently by Meissl (*16*) and by Tangl (*17*) by starving the experimental animals for comparatively short periods and measuring the katabolism by means of a respiration apparatus. The average of their results, computed per 100 pounds in proportion to the two-thirds power of the live weight, is 1,250 calories. The fasting katabolism of swine, computed according to their live weight, is given in Table XII.

TABLE XII.—*Fasting katabolism of swine*

Live weight.	Fasting katabolism per head.	Live weight.	Fasting katabolism per head.
Pounds.	*Calories.*	*Pounds.*	*Calories.*
25	496	300	2,599
50	786	400	3,149
100	1,250	450	3,406
150	1,637	600	4,126
200	1,984		

HEAT INCREMENT DUE TO CONSUMPTION OF FEED BY SWINE

The data regarding the increment of heat production consequent on the consumption of feed by swine are rather meager. Of these, the results of several investigators on grains and on a mixed ration consisting of rice, flesh meal, and whey, as reported by Armsby (*3, p. 656*), are considered trustworthy; and the average of these, 417 calories per pound of dry matter eaten, is taken to represent the energy expenditure by swine.

RATIONS FOR SWINE

The rations and live weights of the animals, upon which the computation of heat emission by swine has been based, are those furnished at the request of Mr. Clarkson, by Prof. J. M. Evvard of Iowa Experiment Station. The weights of the animals and the daily feed eaten, reduced for convenience to dry matter, are the following:

Class.	Weight.	Dry matter of daily feed eaten.
		Pounds.
Suckling pig	25	1.29
Weanling pig	50	2.15
Shote, young	100	3.44
Shote, well-grown	150	6.02
Fattening hog	200	7.74
Farrowing hog	300	6.88
Breeding gilt	300	3.44
Breeding yearling sow	400	4.30
Breeding old sows, year or over	500	5.16
Breeding boar, young	300	3.44
Breeding boar, yearling	450	5.16
Breeding boar, old	600	6.02

TOTAL HEAT PRODUCTION OF SWINE

Since 417 calories represent the increment in heat production by swine per pound of dry matter eaten, the heat increment caused by the consumption of feed by swine is computed on the basis of the foregoing estimates by simple multiplication. To this the fasting katabolism of the animal as found in Table XII is added, and the total heat production is thus obtained (Table XIII).

TABLE XIII.—*Total heat production of typical swine*

Animal.	Live weight.	Total heat emission.
	Pounds.	*Calories.*
Suckling pig	25	1,034
Weanling pig	50	1,684
Shote, young	100	2,684
Shote, well-grown	150	4,147
Fattening hog	200	5,211
Farrowing hog	300	5,468
Breeding gilt	300	4,033
Breeding yearling sow	400	4,942
Breeding old sow	500	5,806
Breeding boar, young	300	4,033
Breeding boar, yearling	450	5,658
Breeding boar, old	600	6,636

HEAT EMISSION BY RADIATION AND CONDUCTION, WATER VAPOR, AND CARBON DIOXID PRODUCED BY SWINE

TABLE XIV.—*Heat emission by radiation and conduction, water vapor, and carbon dioxid produced by typical swine per day and per head*

Animal.	Live. weight.	Heat emission by radiation and conduction.	Latent heat of water vapor.	Amount of water vapor.	Carbon dioxid.
	Pounds.	*Calories.*	*Calories.*	*Gm.*	*Gm.*
Suckling pig	25	776	259	441	414
Weanling pig	50	1,263	421	717	674
Shote, young	100	2,013	671	1,143	1,074
Shote, well-grown	150	3,110	1,037	1,767	1,659
Fattening hog	200	3,908	1,303	2,220	2,084
Farrowing hog	300	4,104	1,367	2,329	2,187
Breeding gilt	300	3,025	1,008	1,717	1,613
Breeding yearling sow	400	3,707	1,235	2,104	1,977
Breeding old sow	500	4,355	1,451	2,472	2,322
Breeding boar, young	300	3,025	1,008	1,717	1,613
Breeding boar, yearling	450	4,169	1,389	2,366	2,223
Breeding boar, old	600	4,977	1,659	2,826	2,654

The results of experiments on man (7) indicate that the heat emission by radiation and conduction is on the average not far from 75 per cent of the total heat emission, and that the relation of the carbon dioxid produc-

tion to the total heat production is likewise about the same as in cattle. The apparent agreement of these relationships between two so widely different species leads to the assumption that the results are, at least for this purpose, applicable also to swine. By using, therefore, the method of computation previously described for cattle, the heat emission by radiation and conduction, the latent heat of water vapor, the amount of water vapor, and the amount of carbon dioxid produced by swine are obtained.

HEAT PRODUCTION OF SHEEP

FASTING KATABOLISM

No direct determinations of the fasting katabolism of sheep are on record. From the rather scanty data regarding the maintenance ration of sheep available Armsby (*3, p. 711*) has computed by an indirect method, based on results with cattle, the fasting katabolism of sheep weighing 100 pounds to be 791 calories, and in proportion to the two-thirds power of live weight as given in Table XV.

TABLE XV.—*Fasting katabolism of sheep*

Live weight.	Fasting katabolism per head.	Live weight.	Fasting katabolism per head.
Pounds.	*Calories.*	*Pounds.*	*Calories.*
20	270	120	890
40	430	140	990
60	560	160	1,090
80	680	180	1,170
100	791	200	1,250

HEAT INCREMENT DUE TO CONSUMPTION OF FEED

No determinations of the increment of heat due to the consumption of feed by sheep have been reported, but it appears probable that the results obtained with cattle (Table II) may be applied to sheep without very serious error.

RATIONS FOR SHEEP

The average rations and live weights upon which the computation of the heat emission by sheep is based were taken from several sources. For fattening lambs, the average ration and live weight given by Henry and Morrison (*10, p. 521*) in their summary of the results on sheep of several different experiment stations were used as an example. For breeding ewes, the average ration and live weight given by Hackedorn (*9*) was used. The average ration for maintenance was taken from Bulletin 143 of the Bureau of Animal Industry, United States Department of Agriculture (*2*), the ration being given in terms of metabolizable

energy, which in a maintenance ration also represents the heat production. The respective live weights and rations per head in the two other cases are as follows:

Fattening lambs, average live weight 81.4 pounds—
Shelled corn, 1.3 pounds.
Alfalfa hay, 1.4 pounds.
Breeding ewes, average live weight 90.3 pounds—
Shelled corn, 0.27 pounds.
Wheat bran, 0.14 pounds.
Linseed meal, 0.05 pounds.
Clover hay, 2.08 pounds.
Silage, 2.09 pounds.

TOTAL HEAT PRODUCTION OF SHEEP

By applying to the foregoing rations the estimates and assumptions of the two preceding paragraphs, the total heat emission for sheep may be computed, and from that the heat emission by radiation and conduction, the latent heat of water vapor, the amount of water vapor, and the amount of carbon dioxid produced, exactly as for the other species. The results are as shown in Table XVI.

TABLE XVI.—*Heat emission, water vapor, and carbon dioxid produced by typical sheep per day and per head*

Animal.	Average live weight.	Total heat emission.	Heat emission by radiation and conduction.	Latent heat of water vapor.	Water vapor.	Carbon dioxid.
	Pounds.	*Calories.*	*Calories.*	*Calories.*	*Gm.*	*Gm.*
Fattening lambs	81.4	2,044	1,533	511	871	818
Breeding ewes	90.3	2,021	1,516	505	860	808
Sheep on maintenance	100.0	1,483	1,112	371	632	593

VENTILATION AND STABLE TEMPERATURE

The method employed by King to compute the rate of air flow for the different species has been given in an earlier paragraph of this paper. It was also shown that, assuming average figures for the percentage of carbon dioxid in the air coming from the lungs and in pure air, respectively, the air flow thus computed corresponds to 0.167 volume per cent of carbon dioxid in the stable air. Taking 0.167 per cent carbon dioxid as a standard, we have computed Table XVII, showing the rate of air flow that will be required to maintain this standard of purity when based on the average computed carbon-dioxid production by the different species in the examples shown in Tables VII, XI, XIV, and XVI. The last column in the table, giving King's figures for the air flow, has been inserted for the sake of comparison.

TABLE XVII.—*Air flow per head to maintain 0.167 per cent carbon dioxid*

Species.	Carbon dioxid produced per day.		Air flow per day.	Air flow per hour.	Air flow per hour according to King.
	Gm.	*Cu. ft.*	*Cu. ft.*	*Cu. ft.*	*Cu. ft.*
Cows	[a] 7,686	138.348	82,843	3,452	3,545
Horses	5,138	92.484	55,379	2,307	4,303
Swine	1,708	30.744	18,410	767	1,394
Sheep	740	13.320	7,976	332	909

[a] Average carbon-dioxid production by cows in milk.

A glance at the last two columns of Table XVII reveals that while King's air flow for cows agrees fairly well with that computed from the carbon dioxid produced, those for other species differ very widely. In other words, either King's figures for volume of respiration in other species than cattle are too high or his assumption of a uniform percentage of carbon dioxid in the expired air is erroneous. At any rate, the actual carbon-dioxid production would seem to be the proper basis upon which to estimate the rate of ventilation required.

TEMPERATURE DIFFERENCE BETWEEN THE STABLE AND OUTSIDE AIR

As already shown, a considerable part of the motive power for stable ventilation is derived from the heat eliminated by the animals, and this heat is also depended upon to maintain the proper temperature in the stable. Obviously, the difference in temperature that can be maintained between the stable air and that outside depends, other things being equal, upon the balance between heat production and heat loss in ventilation. The maximum value of this difference can be computed, when the rate of the air flow and the heat production values are known, on the assumption that no heat is lost by radiation through the walls of the stable. It should, however, be borne in mind that it is only the heat eliminated by radiation and conduction that should be made the basis of the computation, since the latent heat of water vapor, as already explained, is not available for this purpose.

METHOD OF ESTIMATING THE TEMPERATURE DIFFERENCE

Assuming for the purpose of illustration an air movement corresponding on the one hand to King's standard and on the other hand to the amounts computed in Table XVII from the carbon-dioxid production, the temperature difference that can be maintained by the different animals can be determined in the following manner: One calorie of heat can raise the temperature of a pound of water about 4° F. Since the specific heat of air is 0.237 and since 100 cubic feet of air weigh 8 pounds, one calorie of heat can raise the temperature of 100 cubic feet of air $\frac{4}{0.237 \times 8}$, or 2.1°. Consequently the heat required to warm by 1°

the daily volume of air per head required by King's standard or that computed in Table XVII from the carbon-dioxid production is:

Species.	By King's standard	By our estimates.
	Calories.	*Calories.*
Cows	405	394
Horses	492	264
Swine	159	88
Sheep	104	38

Supposing now that all the heat eliminated by the animals by radiation and conduction is imparted to the air passing into and out of the stable—that is, assuming that no heat is lost by radiation through the walls of the stable—this heat is capable of maintaining a temperature difference in degrees Fahrenheit equal to the daily heat emission per head by radiation and conduction divided by the figures of the foregoing table.

MAXIMUM TEMPERATURE DIFFERENCE MAINTAINED BY THE DIFFERENT SPECIES

By the method just described and by the use of the corresponding heat elimination values a calculation has been made of the maximum average difference in temperature between the stable and the outside air that can be maintained by the animal when King's (*14*) standards for ventilation or those computed from the carbon-dioxid production in Table XVII are used. This calculation is given in Table XVIII.

TABLE XVIII.—*Average temperature difference maintained by animals*

Species.	Average live weight.	Average temperature difference.	
		Corresponding to air flow computed by King.	Corresponding to air flow computed from carbon-dioxid production.
	Pounds.	°F.	°F.
Cows[a]	1,075	35.6	36.58
Horses	1,250	19.6	36.49
Swine	280	20.0	36.40
Sheep	91	13.3	36.50

[a] The average heat emission by radiation and conduction by cows in milk were used for the computation.

On the basis of the average figures for the temperature difference given above, Table XIX has been computed, showing approximately the temperature of the stable when air enters at different temperatures,

first, at the rate recommended by King, and second, at the rate computed by the writers (Table XVII, columns 6 and 5, respectively).

TABLE XIX.—*Temperature in stable as compared with that outside*

Temperature of outside air.	Temperature of air in stable.				
	Corresponding to King's figures for ventilation.				Corresponding to air flow given in Table XVII, column 5.
	Cow.	Horse.	Swine.	Sheep.	All species.
°F.	°F.	°F.	°F.	°F.	°F.
−20	15.6	−0.4	0.1	−6.7	16.5
−10	25.6	9.6	10.1	3.3	26.5
0	35.6	19.6	20.1	13.3	36.5
10	45.6	29.6	30.1	23.3	46.5
15	50.6	34.6	35.1	28.3	51.5
20	55.6	39.6	40.1	33.3	56.5
25	60.6	44.6	45.1	38.3	61.5
30	65.6	49.6	50.1	43.3	66.5
35	70.6	54.6	55.1	48.3	71.5

This table is interesting in that it shows approximately the point at which the heat supplied by the animals becomes deficient for maintaining the proper temperature in the stable. Thus, when King's standard of air flow is taken as the minimum, the heat supplied by cows appears to become deficient for maintaining what is believed to be the best stable temperature when the outside temperature is below 15° F. The heat supplied by horses, swine, and sheep appears to become deficient at a much higher outside temperature. When, however, the rate of air flow computed from the carbon-dioxid production is made the basis of the computation, the differences between the species disappear.

MAXIMUM VENTILATION TO MAINTAIN A GIVEN TEMPERATURE DIFFERENCE

Conversely, the same data may be used to compute the maximum rate of air flow compatible with the maintenance of a given temperature difference between the stable and the air outside—for example, a stable temperature of 50° F. in zero weather—since it is evident that if the air flow be reduced the temperature difference and consequently the efficiency of the animals to warm the stable will be increased, while if the ventilation be increased the contrary will be the case. To illustrate this point Table XX has been computed, showing what rate of air movement would be required if it were desired to maintain a temperature difference of 50° between the stable and the outside air. It should be noted that, as in Tables XVIII and XIX, these are maximum values, since no allowance is made for losses of heat through the stable walls.

TABLE XX.—*Maximum rate of air flow possible if a temperature difference of 50° F. is to be maintained*

Species.	Average live weight.	Air flow per day per head.	Air flow per hour per head.
	Pounds.	*Cubic feet.*	*Cubic feet.*
Cows	1,075	60,530	2,522
Horses	1,250	40,459	1,686
Swine	280	13,453	561
Sheep	91	5,825	243

The results recorded in Table XX are notably lower than the minimum air flow computed in Table XVII as necessary to maintain the carbon-dioxid content of the stable air at 0.167 per cent and still further below the rate of air flow recommended by King. In other words (at least in the specific cases used as examples) it will be necessary in severe weather to restrict the ventilation in order to conserve heat and maintain a desirable stable temperature, and consequently the stable air will fall below King's standard of purity—namely, 0.167 per cent of carbon dioxid. The writers are not acquainted with any investigations upon the effects of a higher percentage of carbon dioxid upon animals during long periods, but in experiments with the respiration calorimeter this limit has frequently been exceeded in 2-day trials with no obvious ill effects.

Evidently there is a certain degree of discrepancy between King's requirements and the results of our computations. As has been mentioned on previous pages, the amount of carbon dioxid produced by animals is approximately proportional to their heat production, so that the rate of ventilation should also be approximately proportional to the heat production in order to maintain any desired standard or purity in the air of the stable. It is true that the heat production of animals may vary widely, but to the extent to which the examples we have used may be regarded as typical it appears that the accepted ventilation requirements for the different species are not proportional to their heat production. Taking the values for cows as unity, the relative computed heat production and King's relative ventilation requirements are approximately as follows:

Animal.	Relative heat production.	Relative air movement.
Cows	1.0	1.0
Heavy horses	.8	1.2
Swine	.2	.4
Sheep	.1	.3

Too much stress should not be laid on these differences, in view of the paucity of data for other species than cattle, but they indicate clearly the need for further fundamental investigation.

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TREATMENT OF CELERY SEED FOR THE CONTROL OF SEPTORIA BLIGHT[1]

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Septoria blight is often traceable to the use of infected seed. In a study of control measures at this point an obvious line of attack is through seed disinfection. It is a simple matter to kill spores on the surface of the seed by superficial disinfection. However, many infected seeds have spores in pycnidia embedded in the pericarp, and an attempt to kill these with a chemical disinfectant would endanger the vitality of the seed.

Statements from seed dealers and growers that many celery growers use seed 2 or 3 years old suggested the possibility that the spores of Septoria on and in the seed might not remain viable for so long a period. This indicated a second line of attack.

Accordingly, the problem of control was approached along two lines: (*a*) A study of the effect of aging the seed on the vitality of the spores and mycelium of Septoria; (*b*) the use of hot water as a disinfectant. The results are presented in this paper.

EFFECT OF AGING CELERY SEED ON THE VITALITY OF SEPTORIA SPORES

Conidia taken from celery leaves or from the surface of the seed and stored under the usual laboratory conditions were dead in from 8 to 11 months from maturity. Conidia from the peduncles and pericarps of seed gave a 2 to 3 per cent germination at the end of 2 years but were always dead at the end of 3 years. Spores from 1-year-old seed were slightly more viable than those from 2-year-old seed. Germ tubes of spores from 2-year seed were few and slow in developing, 8 days being the minimum time of appearance. Other results indicate that spores dried for 1 or 2 years in the pericarp of seed lack the vigor to produce germ tubes capable of penetrating the tissues of healthy celery plants. It should be stated that the mycelium in the leaves, on the seed, in the seed, and in the peduncles of the seed was dead at or before the time herein reported for the death of the spores. Therefore, no reference has been made to the age at which the mycelium died. The germination tests of conidia and mycelium were conducted in the field during the

[1] Published with the approval of the director, Massachusetts Agricultural Experiment Station.

The writer is indebted to Prof. A. Vincent Osmun, head of the department of botany of the Massachusetts Agricultural Experiment Station, for criticism of the manuscript.

month of September of three different years, so that environmental conditions might be identical with those of the celery fields.

After determining the maximum age at which spores of Septoria retain their vitality, it was necessary to determine also just how long celery seed remains viable. Seeds of different ages were collected from several reliable seed firms (numbered from 1 to 7 in Table I) and tested to determine the percentage of germination. Two hundred seeds of each year represented under each firm were used in making the tests. These tests were repeated three times during the autumn of 1919 in order to verify results. The percentage of germination given in Table I is the average of the three tests.

TABLE I.—*Percentage of germination of celery seed of different ages* [a]

Seed firm.	5 years old.	4 years old.	3 years old.	2 years old.	1 year old.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	6.0	31.6	34.4	34.6	.....
2	.....	.....	.....	.....	50.2 [b] 47.8
3	.....	35.0	.....	52.0	[b] 41.0
4	11.8	.....	.....	.....	.....
5	.....	32.0	38.8	.....	55.0
6	8.4	35.0	40.2	48.6	.....
7	5.1	40.2	.....	.....	62.5

[a] Blanks indicate that no samples were obtained.
[b] American-grown seed; all other seed French grown.

From Table I it is apparent that celery seed germinates well at from 1 to 4 years of age but that at 5 years the percentage of germination is so reduced that it would be inadvisable to use seed of this age. American-grown celery seed gave a lower percentage of germination than French-grown seed. This explains why in No. 3 the percentage of germination of 1-year-old seed is lower than that for 2-year-old seed. The writer is aware that the percentage of germination of most of the 1-year-old seed presented in Table I is slightly below that of the United States standard of germination (60 to 65 per cent), but since all tests were made under the same conditions the results are relative, and, therefore, admit of comparison.

By purchasing seed 1 or 2 years old and retaining it until it is 3 or 4 years old the grower not only eliminates the fungus but has the opportunity to test the seed for pureness of strain, quality of plant produced, and elimination of seed weak in vitality.

HOT-WATER METHOD OF SEED DISINFECTION

It can be readily seen that any method of surface disinfection is insufficient to destroy the spores embedded in pycnidia in the pericarp of the seed. Other investigators have devised various means of meeting

this problem. Coons and Levin [1] recommend soaking the seed in warm but not hot water for one-half hour and then in a 1 to 1,000 corrosive-sublimate solution for one-half hour. Watts [2] states that some growers disinfect their seed by placing it for a few minutes in a 1 to 32 copper-sulphate solution. The seed should be dried thoroughly after this treatment.

The method devised by the writer is based on tests to determine whether the thermal death point of the fungus is lower than that of celery seed. Thermal exposures (30 minutes was substituted for the usual 10) were made of the pathogene under the following conditions: (a) Spore suspensions in sterile distilled water in test tubes; (b) mycelium 2 weeks old in sterile distilled water in test tubes; (c) nutrient agar cultures 2 weeks old in test tubes. These forms of the fungus were heated in a double boiler for 30 minutes to each degree of temperature between 38° and 55° C. The results showed that only a slight percentage of the spores were viable after being heated to 38° and that none retained viability after exposure at 40°. None of the nutrient agar cultures were viable at 44°, whereas the mycelium in the water blanks was nonviable at 45°. The higher death point of mycelium in the water blanks is probably due to the different densities of the two media.

The method adopted for determining the thermal death point of the seed is as follows: Duplicate lots of 200 seed of Golden Self-blanching celery inclosed in cheesecloth bags were heated in water at 40° C. for 30 minutes, and at each degree between 45° and 50°. Untreated controls for comparison were used in all cases. The results are shown in Table II.

TABLE II.—*Percentage of germination of celery seed heated at various temperatures*

Temperature.	Percentage of germination of seed.	Temperature.	Percentage of germination of seed.
°C.		°C.	
40	49.5	48	44.0
45	51.8	49	40.0
46	43.3	50	32.8
47	44.5	Control.	46.8

In other words, the temperatures of 40° and 45° C. increased germination 5.8 and 10.7 per cent, respectively, over the control, whereas the temperatures from 46° to 49° injured germination slightly. At 50° germination fell 29.9 per cent below the control. The temperature at which celery seed loses its vitality lies somewhere between 50° and 55°. From the foregoing it is evident that heating 1- and 2-year-old celery seed in water at 48° or 49° for 30 minutes eliminates all possibility of Septoria infection of seedlings from such seed.

[1] COONS, G. H., and LEVIN, Ezra. THE SEPTORIA LEAF SPOT DISEASE OF CELERY OR CELERY BLIGHT. Mich. Agr. Exp. Sta. Spec. Bul. 77, 8 p., 9 fig. 1916.

[2] WATTS, Ralph L. VEGETABLE GARDENING. p. 302–326. New York. 1912.

SUMMARY AND CONCLUSIONS

As a rule American-grown celery seed is freer from Septoria spores than French-grown seed.

Spores and mycelium taken from the surface of celery seed or from the pycnidia in celery leaves and kept in the laboratory lose their vitality in 8 to 11 months.

Conidia and mycelium from pycnidia in the peduncles and pericarp of celery seed gave from 2 to 3 per cent germination at the end of 2 years but were dead at the end of 3 years.

The vitality of the conidia and the mycelium which germinate at the end of 1 and 2 years is very low.

Celery seed 3 or 4 years old gave a good germination test. The percentage of germination of seed more than 4 years old is too low to warrant the use of such seed by commercial growers.

The conidia are killed when heated for 30 minutes in water at 40° C.; the mycelium is killed at 45°; celery seed when similarly heated is not appreciably injured below 50°. The vitality of celery seed is destroyed between 50° and 55°.

The vitality of spores and mycelium in the pericarp of celery seed is destroyed at 48° and 49° C. without seriously injuring the germinating power of the seed; higher temperatures greatly impair the power of the seed to germinate.

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BIOLOGY AND ECONOMIC IMPORTANCE OF ANASTATUS SEMIFLAVIDUS, A RECENTLY DESCRIBED EGG PARASITE OF HEMILEUCA OLIVIAE

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INTRODUCTION [1]

Adults of the recently described egg parasite *Anastatus semiflavidus* Gahan were first reared from eggs of the New Mexico range caterpillar, *Hemileuca oliviae* Ckll., during the progress of experiments to determine methods for the economic control of that destructive pest.

These rearings were made by F. H. Gates from *Hemileuca oliviae* eggs collected in the vicinity of Koehler, N. Mex., during June, 1913. For several years prior to this date extensive egg collections in the same vicinity and throughout the infested area had failed to reveal the presence of any egg parasite of *H. oliviae*.[2] It is assumed from the foregoing that, just before the period during which these negative collections were made, some severe and unusual climatic condition had reduced the numbers of the parasite to such an extent that its presence was not discovered at that time. Since its discovery in 1913 the parasite has appeared in increasing numbers each year until it now exerts a powerful influence in the natural control of its host.

DISTRIBUTION OF THE PARASITE

Egg collections made during the autumn of 1915 and the winter and spring of 1916 demonstrated that the parasite was widely distributed in that part of northeastern New Mexico which was heavily infested by the range caterpillar. Adults were reared from the following localities in that section: Maxwell, Brackett, Nolan, Las Vegas, Springer, Chico, Cimarron, Mora, Watrous, Miami, Colfax, Folsom, Wagon Mound, Vermejo, Koehler, Colmor, Taylor, Levy, Roy, Mills, and Clayton.

[1] The observations detailed in this paper were made principally at the United States Range Caterpillar Camp near Koehler, N. Mex., and at the United States Entomological Laboratory, Maxwell, N. Mex. V. L. Wildermuth, F. H. Gates, W. F. Schlupp, and H. E. Smith assisted in securing the data herein presented.

[2] AINSLIE, C. N. THE NEW MEXICO RANGE CATERPILLAR (HEMILEUCA OLIVIAE CKLL.). *In* U. S. Dept. Agr. Bur. Ent. Bul. 85, pt. 5, p. 59–96, fig. 32–53, pl. 3–4. 1910.

CLASSIFICATION AND DESCRIPTION

Anastatus semiflavidus belongs to the hymenopterous superfamily Chalcidoidea, family Encyrtidae, and subfamily Eupelminae. It was described as a new species by A. B. Gahan,[1] of the Bureau of Entomology, from type specimens reared from *Hemileuca oliviae* eggs by F. H. Gates at Koehler, N. Mex. Mr. Gahan's description of the adult follows.

ADULT

Anastatus semiflavidus, new species. *Female* [Pl. 68, A].—Length, 2.3 to 2.5 mm. Head strongly punctate; eyes elliptical; antennal pedicel about two-thirds the length of the first funicle joint; ring-joint transverse; first, second, and third funicle joints subequal, following joints shorter; mesoscutum with the median and lateral lobes alike faintly scaly-punctate and hairy; the median lobes more distinctly sculptured bordering the lateral margins; scutellum and axillae very finely and closely punctured, the former precipitous posteriorly and the posterior face smooth; propodeum smooth; mesopleurae mostly smooth, but with the anterior portion above scaly-punctate; postmarginal vein twice as long as the stigmal, the marginal a little more than twice the postmarginal; abdomen faintly lineolate, about as long as the thorax. Scape reddish-yellow, flagellum black; head brassy-green; mesoscutum, punctate area on the mesopleurae, posterior face of the scutellum, propodeum, hind coxae, and underside of the thorax metallic blue-green; scutellum and axillae varying from wholly pale orange-yellow to dark brown, with only the bases yellowish; remainder of the thorax reddish yellow; legs yellowish within and along the margins, blackish or brownish outwardly, the femora often tinged with metallic; wings fuscous, the base hyaline to the beginning of the marginal vein and a broad hyaline transverse band before the stigmal vein; abdomen yellowish above except the three apical segments, which are darker and somewhat metallic; venter pale at base, brownish medially and metallic apically.

Male [Pl. 68, B].—Head strongly punctate; antennal scape compressed and expanded beneath, pedicel very short, flagellum tapering slightly from base to apex; first funicle joint about twice as long as wide; following joints successively shortening; club scarcely as long as the two last funicle joints combined; mesoscutum and scutellum alike scaly-punctate, mesopleurae mostly smooth; propodeum smooth; postmarginal vein nearly as long as the marginal and a little more than twice the length of the stigmal; abdomen reticulately lineolate. Color dark blue-green; antennae black, the expansion of scape pale; abdomen beyond the first segment brownish-black; all trochanters, a line above and the apices of front and middle femorae, front tibiae outwardly for its whole length, basal third of middle and hind tibiae and the three basal joints of the middle and hind tarsi yellowish white; front tarsi and apical two joints of the other tarsi fuscous; remainder of the legs blue-green or blackish.

Type-locality.—Koehler, New Mexico.

Host.—*Hemileuca oliviae.*

Type.—Cat. No. 18331, U. S. N. M.

LARVA

The freshly dissected larva (fig. 1) is dirty white in color with the body contents showing darker. When viewed dorsally, the general shape is elliptical, becoming slightly broader posteriorly. When viewed later-

[1] GAHAN, A. B. DESCRIPTIONS OF NEW GENERA AND SPECIES, WITH NOTES ON PARASITIC HYMENOPTERA. *In* Proc. U. S. Nat. Mus., v. 48, p. 155–168. 1914.

ally, the larva appears crescent-shaped and is slightly depressed dorso-ventrally. The head is partly retracted and not chitinized. The mandibles are small and almost invisible. The body segments, 13 in number, are well defined and slightly broader dorsally than ventrally. This causes a well-defined fold just above the pleural areas which becomes more pronounced posteriorly. A narrow ridge extends down the median line dorsally. The head and first two body segments are sparsely pubescent.

FIG. 1.—*Anastatus semiflavidus:* Larva, side view. Greatly enlarged.

The average dimensions of the larva are: Length when extended, 2.5 mm.; width, 1.25 mm.; depth dorso-ventrally, 1 mm.

PUPA

The pupa (fig. 2) when first formed is creamy white in color and closely resembles the adult in shape. The appendages are folded close to the body, and the entire pupa is covered by a thin pupal skin. The sex differences are early apparent. The female pupa, when extended, averages 2.5 mm. in length, and the male pupa, 1.75 mm. Soon after formation, the eyes of both sexes turn pink and gradually the thorax and abdominal bands of the female become light brown in color, while in the male they become dark blue-green. Just before the emergence of the adult, the entire pupa assumes the characteristic color of its sex.

FIG. 2.—*Anastatus semiflavidus:* Pupa, side view of female. Greatly enlarged.

LIFE HISTORY

EGG PERIOD

All attempts to dissect the egg of *Anastatus semiflavidus* from its host or to ascertain the length of the incubation period have been unsuccessful.

The period of normal oviposition in the field begins when the *Hemileuca oliviae* eggs are first deposited, about the middle of September, and continues until the arrival of severe winter weather, which occurs in these comparatively high altitudes during late November or early December.

Judging from indications furnished by numerous dissections of host eggs from life-history cages and from eggs collected at various times in the field, it is probable that the parasite eggs which are deposited just before the beginning of cold weather hibernate in that stage, while the eggs which are deposited early in the season hatch and hibernate as

partly developed larvæ within their host. This theory would account for the presence of the fully developed larvæ that are frequently found within their host eggs early in the succeeding spring.

LARVAL PERIOD

Periodic dissections and emergence records of egg clusters that had been exposed to adults in confinement indicated extreme variations in the duration of the larval period for different individuals.

From a series of isolated host egg clusters that had been exposed to parasite adults in cages for two days during the last week of September, 1914, a few adults emerged early in May, 1915. The larval period of these individuals therefore was limited to about seven months, whereas some of the other eggs in these same egg clusters were found to contain full-grown dormant larvæ nearly two years later—that is to say, in March, 1917.

Dissections of 1- and 2-year-old eggs collected in the field gave similar results and established the fact that on some occasions the insect remains in a dormant larval state for at least two years.

It will be apparent from the foregoing that any statement concerning the duration of the larval period must necessarily be in approximate terms.

PUPAL PERIOD

The pupal period is evidently very short, as in a long series of dissections of parasitized eggs, made at intervals of three or four days, it was observed that pupæ were rarely found, although many of these eggs contained fully developed larvæ and adults were emerging each day.

Pupæ were dissected out of the host and placed in different types of cages in an attempt to ascertain the length of the pupal period, but none were reared through to the adult form.

ADULT PERIOD

In laboratory experiments the females remained alive for several weeks in Doten cages that were supplied with equal parts of honey and water as food. Under these same conditions the males remained alive but a few days after being placed in the cages.

Adults have been observed in the field from the early part of May until the first of December, the maximum emergence occurring during July and August. As the host eggs are not deposited until September, it is probable that the females live for long periods under field conditions, the length of their life depending upon the proximity of their host and upon the weather conditions.

DURATION OF LIFE CYCLE

From a series of 49 cages started in September and October, 1914, a total of 383 adults were reared. These adults required a maximum of

449 days and a minimum of 226 days to complete their life cycle, the average being 380 days. (See Tables I and II for a record of two of these cages.) From a series of cages started in October, 1915, a total of 40 adults were reared. These adults required a maximum of 346 days and a minimum of 238 days to complete their life cycle, the average being 266 days. From a series of stock cages started the last week in September, 1914, adults were reared from the first week in the following May until March, 1917, a minimum of 7 months and a maximum of 30 months. It is evident that this prolonged life cycle was not due to the artificial cage conditions because adults emerged from 1- and 2-year-old eggs collected in the field.

TABLE I.—*Duration of life cycle, proportion of sexes, and progeny of one female Anastatus semiflavidus*

Cage No.	Date egg was exposed.	Date adult parasite emerged.	Total number of days.	Number and sex of adults.	Result of dissecting eggs still intact on May 20, 1916.
	1914.	1915.			
817 (1)	Sept. 17	Sept. 22	370	2 ♂, 1 ♀	
		26	374	1 ♀	One living larva.
		30	378	1 ♀	Two dead larvæ.
		Oct. 26	404	2 ♀	
		Dec. 4	443	1 ♀	
817 (2)	18	Sept. 22	369	1 ♂, 2 ♀	One living larva.
817 (3)	20	Oct. 22	397	1 ♀	Do.
		Dec. 10	446	1 ♀	One dead female.
817 (4)	22	Sept. 3	344	1 ♂	
		22	365	2 ♂	One living larva.
		24	367	2 ♀	
817 (5)	25	24	364	1 ♀	
		25	365	1 ♀	Do.
		Oct. 19	390	1 ♀	One dead larva.
		Nov. 16	418	1 ♀	Do.
		20	422	1 ♀	
817 (6)	27	May 17	233	1 ♂	
		Sept. 22	360	3 ♀	
		25	363	2 ♀	
		26	364	1 ♀	One living larva.
		30	369	1 ♀	
		Oct. 2	371	1 ♀	
		4	373	1 ♀	
817 (7)	29	Sept. 22	358	2 ♂	
		24	360	1 ♂	
		26	362	2 ♀	One dead larva.
		30	366	2 ♀	
		Oct. 25	392	1 ♀	
817 (8)	Oct. 1	Sept. 7	342	1 ♂	

Average number of days in life cycle = 373.4.
Maximum number of days in life cycle = 446.
Minimum number of days in life cycle = 233.
Proportion of sexes = 12 males and 32 females.
Total progeny of one female = 54 individuals.

Many experiments were carried on to determine whether this parasite would reproduce in mature eggs. No reproduction was secured in any of these experiments.

The extreme variation in the duration of the life cycle, as shown in Tables I and II, is important in that it would allow the parasite to survive any unfavorable weather conditions for an extended period.

TABLE II.—*Duration of life cycle, proportion of sexes, and progeny of one female Anastatus semiflavidus*

Cage No.	Date egg was exposed.	Date adult parasite emerged.	Total number of days.	Number and sex of adults.	Result of dissecting eggs still intact on May 20, 1916.
	1914.	1915.			
818 (1)	Sept. 18	July 24	309	1 ♂	Three dead larvæ.
		Aug. 1	316	1 ♂	
		24	339	1 ♂	
		Dec. 7	445	1 ♂	
818 (2)	20	Sept. 22	367	2 ♀	
		24	369	1 ♀	
		27	372	1 ♀	
		30	375	1 ♀	
		Oct. 8	383	1 ♀	
818 (3)	22	.....	.....	.....	No parasitism.
818 (4)	25	Sept. 22	362	1 ♂	One dead larva.
		24	364	1 ♂, 2 ♀	One dead male.
		Dec. 9	440	1 ♂	
818 (5)	27	Sept. 24	362	2 ♀	
		Oct. 17	385	1 ♀	One dead female.
		26	394	1 ♀	
818 (6)	29	Sept. 22	358	1 ♂, 1 ♀	
		24	360	1 ♀	
		25	361	1 ♂, 1 ♀	One living larva.
		26	362	1 ♀	
		Oct. 10	376	1 ♂	
		22	388	1 ♂	
818 (7)	Oct. 1	Sept. 30	364	1 ♂, 1 ♀	
		Oct. 8	372	1 ♀	
		12	376	1 ♂	One dead larva.
		21	385	2 ♂, 1 ♀	
		Nov. 7	402	1 ♂	
818 (8)	3	May 17	226	1 ♂	Four dead larvæ.
		Sept. 3	335	1 ♀	One dead pupa.
		22	354	3 ♀	Two dead females.
		25	357	1 ♀	
		Nov. 7	400	1 ♂	
818 (9)	5	Sept. 28	358	1 ♀	
		Oct. 25	385	1 ♂	

Average number of days in life cycle = 366.7.
Maximum number of days in life cycle = 445.
Minimum number of days in life cycle = 226.
Proportion of sexes = 20 males and 29 females.
Total progeny of one female = 60 individuals.

CAGE REARING METHODS

The cages used in experiments with *Anastatus semiflavidus* adults were of a modified Doten type.[1] This cage consisted of two round 30 by 100 mm. glass vials, of the same diameter and shape, cut off squarely at the open ends. A small patch of beeswax was melted on the inside of one vial, at a point equidistant from each end, to serve as a support for the food of the Hymenoptera. This food consisted of equal parts of honey and water and was applied as a small drop in a depression made in the beeswax. This vial was known as the food tube. The other vial was known as the home tube and was left in its original condition.

The Hymenoptera were introduced into the home tube, and the open ends of the two vials were placed together. A strip of heavy wrapping paper, 2 inches wide and 10 inches long, was then bandaged tightly around the abutting edges and secured by snapping a heavy rubber band around each vial at the edge of the paper bandage.

When it became necessary to change the food tube for replenishment or cleaning, a duplicate food tube was prepared to replace the original. Before this change could be affected, however, it was necessary to entice the Hymenoptera into the other, or home tube. This was accomplished by holding the home tube with its closed end toward the light and inclined slightly upward. A smart tap on the bottom of the food tube would usually drive any insects into the home tube that refused to crawl out. When all the Hymenoptera in the cage had moved into the home tube, the rubber band holding the original food tube was removed. The other rubber band on the home tube held the paper bandage in position while the newly prepared food tube was quickly inserted and its rubber band replaced.

In life-history experiments it became necessary to introduce egg clusters of the host into these cages. Under these circumstances, the Hymenoptera were enticed into the food tube, after which the home tube was removed, the host egg cluster quickly inserted, and the home tube returned to its original position.

The glass sides of this cage made it possible to follow easily the activities of the insects under observation, and its mobility allowed it to be placed upon the binocular stage for microscopic examination of its contents.

The labels and other necessary data were written on the paper bandage holding the two vials together.

The cage was prevented from rolling and smearing the honey solution over the interior by placing it in a shallow pasteboard box with loose cotton.

[1] DOTEN, Samuel B. CONCERNING THE RELATION OF FOOD TO REPRODUCTIVE ACTIVITY AND LONGEVITY IN CERTAIN HYMENOPTEROUS PARASITES. First paper. Nev. Agr. Exp. Sta. Tech. Bul. 78, p. 8–10, pl. 1. 1911.

Adults of *Anastatus semiflavidus* were kept alive for long periods in this type of cage, provided proper care was exercised in its maintenance. The honey solution was kept fresh, a new supply being prepared every few days, as experience demonstrated that a rancid or moldy condition of the food caused the death of the Hymenoptera. The interior of the cage was kept strictly clean and free from all particles of dirt or other foreign matter that would entangle the insects.

The direct rays of the sun proved fatal to the confined insects, so it was necessary to keep the cages in a shaded location.

HABITS

FERTILIZATION

The union of the sexes and the fertilization of the female take place in the manner common to most chalcids soon after the adults emerge and last but a few seconds.

When this species was first reared from *Hemileuca oliviae* eggs, it was thought that the male and female *Anastatus semiflavidus* were separate species. Each sex was accordingly exposed to eggs of *H. oliviae* in separate cages for the purpose of securing life-history records. Under these conditions, the species proved parthenogenetic, as all the progeny of the unmated female *A. semiflavidus* were males.

OVIPOSITION

The process of oviposition was often observed in life-history cages. In one instance, a female of *Anastatus semiflavidus* was introduced into a glass vial cage containing a *Hemileuca oliviae* egg cluster known to have been deposited 2 days previously. This female had been confined in a stock cage containing many adults of both sexes and had probably been fertilized. It had not previously been exposed to any eggs of its host. Immediately upon being introduced into the cage, the female began examining the egg cluster, running over the surface of the eggs and feeling them with her antennæ. After 2 or 3 minutes she apparently selected an egg that suited her purpose and began making preparations to oviposit. V. L. Wildermuth then took this female under observation and through the binocular microscope observed the details of the process of oviposition. He states that in the two instances under his observation the female drilled a hole in the shell of the egg with her ovipositor in 15 and 20 minutes, respectively, after which an egg was apparently deposited in each instance. Mr. Wildermuth then directed the writer to observe the details of oviposition and to describe the same. After a short interval this cage was again examined by the writer, and soon the female was observed making preparations to oviposit.

Through the binocular microscope, it was seen that the female was poised over the selected egg, the legs being strongly braced against the sides of the adjoining eggs, and most of the pressure being exerted

through the posterior pair of legs. The ovipositor protruded a short distance (fig. 3), and the abdomen moved slowly up and down, forcing the ovipositor into the shell of the egg. Occasionally the movement was from side to side, and there were short intervals of rest.

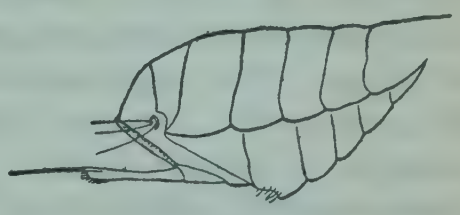


FIG. 3.—*Anastatus semiflavidus:* Side view of posterior segments of female abdomen with extruded ovipositor. Greatly enlarged.

After 21 minutes, the opening in the egg appeared to be completed and a small drop of brownish liquid appeared at the point where the ovipositor was inserted in the egg. It is impossible to state whether this liquid exuded from the female or came from the egg. When this operation of drilling an opening in the egg was complete, the female turned around and examined the puncture. She appeared to be sipping the liquid around the opening in the eggshell or possibly feeding on the contents of the egg. The female soon resumed her original position and inserted the ovipositor into the egg, nearly full length. The ovipositor was then partly withdrawn and thrust again into the egg. This operation was repeated 9 or 10 times. Finally the ovipositor was thrust in full length, the female crouched low over the egg, the abdomen, meanwhile, was drawn sharply forward, and oviposition apparently took place. The female remained quiet, except for a slight movement of the abdomen, in this act of oviposition for 2 or 3 minutes. The ovipositor was then withdrawn, and the female turned around and manipulated the mandibles and antennæ in the small drop of liquid which remained over the opening in the egg. This liquid soon hardened and formed a transparent, light brown, waxy substance which apparently sealed the oviposition puncture. After completing oviposition in this egg, the female resumed her examination of the remaining eggs in the cluster and was observed to oviposit several times during the remainder of the day.

INTERNAL APPEARANCE OF PARASITIZED EGG

For a long period after oviposition the internal appearance of a parasitized egg does not differ from the normal, the contents consisting of a reddish brown liquid. The only change apparent, until the parasite larva becomes visible, is a slight thickening of the liquid contents. In the course of its development, the larva consumes these liquid contents and finally occupies about two-thirds of the egg-cavity. Only one larva develops in each egg.

EXTERNAL APPEARANCE OF PARASITIZED EGG

Superficially the external appearance of a parasitized egg does not differ from the normal, there being no change in its color or shape. Microscopic

examination, however, reveals the presence of the sealed oviposition puncture, and this is the only external indication by which a parasitized egg may be recognized. The parasite can not be discerned, even during its advanced stages, through the opaque, thick-walled shell of the egg. This appearance is in marked contrast to that of eggs of the gypsy moth (*Porthetria dispar* L.) which have been parasitized by *Anastatus bifasciatus* Fonsc. The parasite larva of this closely allied species is plainly visible through the shell of its host egg.

Hemileuca oliviae larvæ frequently hatch from eggs bearing oviposition punctures of the parasite. This is probably due to the fact that these particular eggs were not successfully parasitized.

APPEARANCE OF HOST EGGS FROM WHICH ADULTS HAVE EMERGED

The adult, upon emerging from the pupa, finds itself completely inclosed within the walls of the host egg. It gnaws a small but easily distinguishable hole through this wall and makes its escape.

The host eggs from which *Anastatus semiflavidus* adults have issued may be readily recognized by the presence of this exit hole. It is much smaller than the hole made by the hatching of the host larvæ. (Pl. 68, C.)

METHOD OF LARVAL DEVELOPMENT

The larva of *Anastatus semiflavidus* prevents the formation of the embryonic larva of its host and feeds exclusively upon the liquid contents of the host egg. This method of larval development is characteristic of the true egg parasites.

DORMANT PERIOD OF FULL-GROWN LARVA

After the larva has become full-grown, the development of the pupal stage depends largely upon the external climatic conditions to which the egg is subjected. In northern New Mexico, these climatic conditions refer especially to humidity and must be considered in this special sense. The degree of humidity as affecting the development of parasite larvæ in this semiarid climate depends largely upon whether the eggs are situated on high or low ground, or upon the distance from areas of surface water.

If the parasitized egg, containing a full-grown larva, happens to be subjected to a long period of drought, pupation may be delayed and a dormant larval period of indefinite duration produced until such a time as both humidity and temperature are favorable for further development.

When these favorable conditions occur, the full-grown dormant larva changes to a pupa without regard to the length of time spent in the larval stage.

SHORT LENGTH OF PUPAL PERIOD

The duration of the pupal period is very short, as noted by numerous dissections. From this fact it is assumed that the insect must be less

resistant to adverse weather conditions as a pupa than as a dormant larva or as an adult, and it would appear that the pupal period is made as short as possible in order to hurry the insect through the most vulnerable period of its existence. The few pupæ dissected from parasitized eggs have invariably been found during periods of heavy adult emergence.

NUMBER OF INDIVIDUALS PRODUCED BY EACH FEMALE

In cage experiments, two females under observation produced 60 and 54 individuals, respectively (Tables I and II). These figures may be considered as being below the average, because under natural conditions each female probably produces a greater number of progeny than when confined in cages.

JUMPING HABIT OF ADULTS

Although equipped with wings, the adults of both sexes appear to lack the power of sustained flight. Locomotion seems to be accomplished largely by means of jumping or running. Adults that were observed in the field running around on the ground in the vicinity of host egg clusters disappeared with startling rapidity when an attempt was made to collect them, their movements, when approached, resembling those of the halticine flea-beetles. This ability to jump made the species very difficult to handle in the laboratory cages, and many adults were lost during the process of feeding or when they were being transferred from one cage to another.

The males are much more active than the females.

RELATIVE PROPORTION OF SEXES

From a quantity of *Hemileuca oliviae* eggs collected in the field a total of 393 adults issued. Of this number 158 were males and 235 were females. From two series of life-history cages a total of 423 adults issued. Of this number 175 were males and 248 were females. These figures would seem to indicate that both sexes were well represented but that the females were slightly more abundant than the males. (Tables I and II.)

POSITIVELY PHOTOTROPIC HABIT OF ADULTS

The adults of *Anastatus semiflavidus* are positively phototropic. This characteristic was used to advantage in rearing the adults from eggs collected in the field and in handling the species in life-history cages.

HOSTS OTHER THAN HEMILEUCA OLIVIAE

Adults of *Anastatus semiflavidus* were reared from eggs of *Hemileuca nevadensis* Stretch, collected from willow along the banks of the Red River in New Mexico. Laboratory experiments also demonstrated that

adults of *A. semiflavidus*, emerging from *H. oliviae* eggs, would breed in eggs of *H. nevadensis*.

The eggs of this closely allied species are not very numerous in the region infested by *Hemileuca oliviae* and, therefore, do not form a very important host for *Anastatus semiflavidus*. If for any reason, however, *H. oliviae* should become greatly reduced in numbers, the eggs of *H. nevadensis* would serve as a valuable host in perpetuating *A. semiflavidus*. From many indications, it is probable that such a condition of affairs has existed in the past. A study of the life history of *H. nevadensis* demonstrated that its life cycle and that of *H. oliviae* correlate very closely.

An attempt was made to rear *Anastatus semiflavidus* from the eggs of *Malacosoma fragilis* Stretch, collected from scrub oak along the foothills, and to secure parasitism of these eggs in confinement, but only negative results were obtained.

ECONOMIC IMPORTANCE OF THE PARASITE

It has been very noticeable that this highly beneficial insect has been increasing rapidly in numbers since the spring of 1913 when the species was first discovered. This increase has been especially marked in areas of heavy *Hemileuca oliviae* infestation.

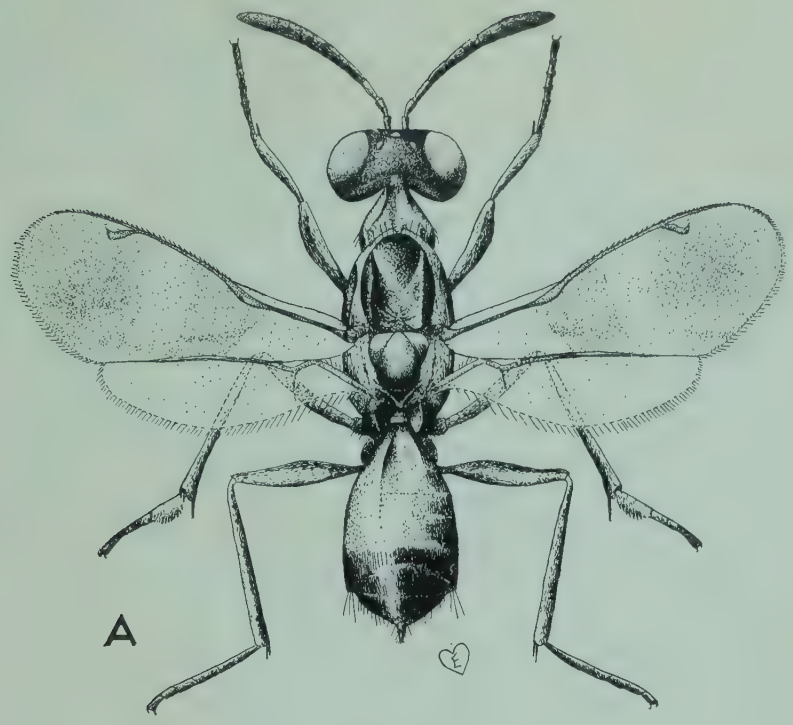
In the collections of *Hemileuca oliviae* eggs made from different localities during the autumn of 1915 and the winter and spring of 1916 the percentage of parasitism varied from 75 to only a trace. The species was found to be present in every locality from which *H. oliviae* eggs were collected with the exception of two isolated areas far to the south of the main area of infestation, in which only a very few egg clusters were found.

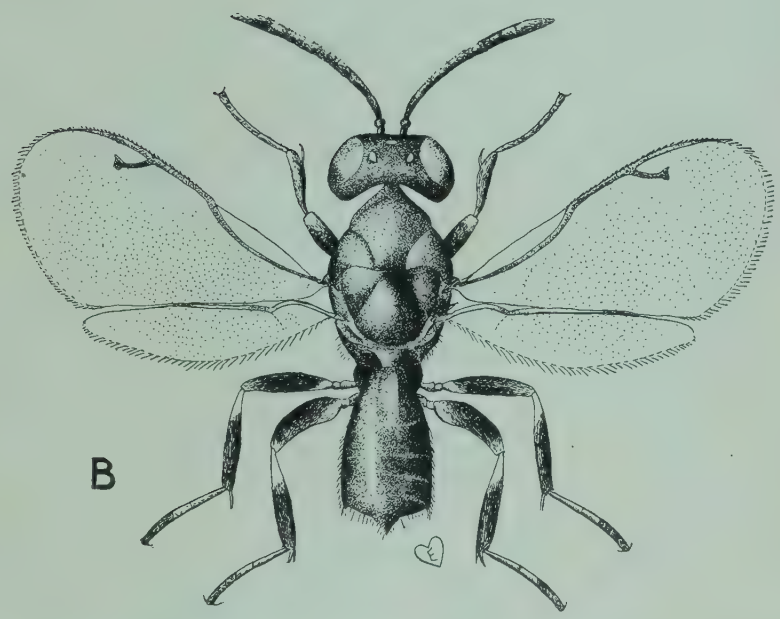
At the present time *Anastatus semiflavidus* appears to be one of the most efficient natural enemies contributing to the control of *Hemileuca oliviae*.

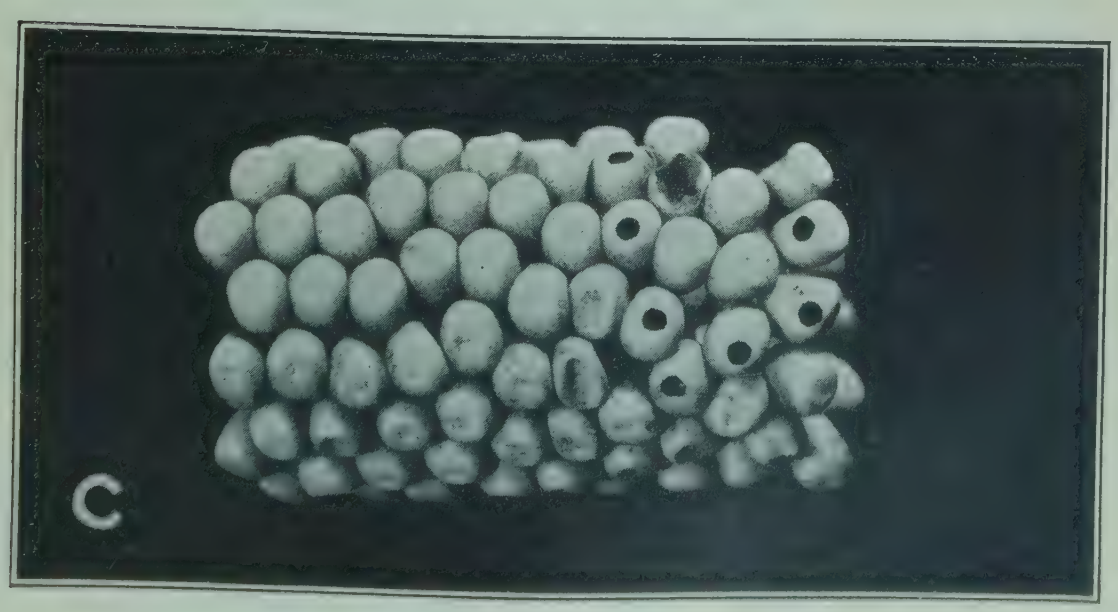
PLATE 68

Anastatus semiflavidus:

A.—Adult female, dorsal view.
B.—Adult male, dorsal view.
C.—Exit holes of adults from egg clusters of *Hemileuca oliviae.*


A


B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VARIETAL SUSCEPTIBILITY OF BEANS TO RUST [1]

By F. D. FROMME, *Plant Pathologist*, and S. A. WINGARD, *Assistant Plant Pathologist*, *Virginia Agricultural Experiment Station*

INTRODUCTION

The bean rust fungus, *Uromyces appendiculatus* (Pers.) Fries, is worldwide in distribution, being found practically coextensive with the distribution of its hosts throughout the North American continent and in the West Indies, South America, Europe, Africa, Asia, Japan, and Australia. In addition to its common host, *Phaseolus vulgaris* L., it occurs in North America on ten species of Phaseolus, two species of Dolichos, two of Strophostyles, and three of Vigna (*1*).[2] It is an autoecious species with the full complement of spore forms. The aecia are of the cupulate type and are of rather rare occurrence, judging from the collections in herbaria. The writers have seen them in the field on only one occasion. The uredinia and telia are found during the summer and fall and are very common. The relative importance of the different spore forms in the overwintering of the rust has not been determined, but it seems likely that the teliospores are of minor importance, at least under Virginia conditions, and that the urediniospores are the most important factor in overwintering.

In the opinion of the writers bean rust is commonly underrated as a limiting factor in bean production. The losses are indirect, being occasioned by decreased vigor due to foliage injury, and this fact tends to obscure the amount of curtailment of yield except in very severe cases. Although the disease occurs in all parts of the United States losses seem to be especially severe in Virginia, West Virginia, Tennessee, and southern Ohio of the eastern States, in Georgia and Louisiana of the southern States, and in California. In the commercial bean areas of the North it seemingly appears too late as a rule to cause much injury. It is occasionally severe in the New England States. In Virginia bean rust is one of the chief limiting factors in production. Severe injury is commonly found on three varieties, Kentucky Wonder, Navy Pea, and Tennessee Green Pod. Evidence of the severity of bean rust in California and of the inefficacy of spraying may be seen in the following quotations. R. E. Smith (*10*) in discussing the work at the California Experiment Station says:

> Mr. Rogers did a considerable amount of work during the year in an endeavor to find means for checking a serious rust (*Uromyces appendiculatus*) which attacks the

[1] Published with the approval of the Director of the Virginia Agricultural Experiment Station. Paper 56 from the Department of Plant Pathology and Bacteriology.

[2] Reference is made by number (italic) to "Literature cited," p. 404.

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Key No. Va. (Blacksburg)-3

Kentucky Wonder bean in San Diego county and prevents the growing of this valuable crop late in the fall. Treatment both with Bordeaux mixture and finely ground sulfur was tried, but unfortunately neither material gave any appreciable control of the disease.

D. C. Milbrath [1] is quoted as follows:

In Los Angeles county, especially in the San Gabriel Valley, rust developed so rapidly on beans during the past week that some fields have been abandoned. This is the case particularly with Lady Washington and French varieties.

Until recently there has been little specific information in literature on the relative susceptibility of bean varieties to rust.

Duggar (*2*) states that great differences exist in the susceptibility of varieties among both dwarf and pole sorts and suggests that the selection of resistant varieties would seem to be possible.

Gassner (*5*) noted a difference in the susceptibility of 2 varieties of beans in South America and later planted 15 varieties in a field test. These were inoculated with rust spores, and wide variations in susceptibility were seen. Three of the varieties were severely injured, while the remainder were only moderately, slightly, or not at all affected.

Jordi (*7*), in Europe, took field records on five varieties of pole beans with respect to severity of rust, using the cipher 1–10 to indicate the state of susceptibility. Four of the varieties were susceptible, being rated 7–10, while one, Klosterfrauen, was resistant, being rated only 0.5–1.

In the Plant Disease Bulletin for September 15, 1918, W. J. Morse (*9*) lists 17 varieties of beans with respect to their susceptibility to rust in Maine. Seven of the varieties were severely attacked, while the other 10 developed only slight infection or none.

In a previous publication (*4*) the writers have described the field behavior of a large number of bean varieties with respect to their susceptibility to rust. Very marked variations were found together with great curtailment of productivity, some of the most susceptible varieties being complete failures.

It is the purpose in this paper to describe more fully the various phenomena found in studies of rust infection and development on certain varieties of beans, especially under greenhouse conditions.

EXPERIMENTAL METHODS

Considerable preliminary work was devoted to the development of a technic. A standard dosage, method of inoculation, and uniformity in the records of infection were recognized as some of the first requisites. One of the greatest difficulties in this connection lies in the material itself. The pathogene, together with other Uredinales, can not be grown apart from the living host, and in consequence the pure culture

[1] MILBRATH, D. C. [FIELD NOTES.] *In* U. S. Dept. Agr. Bur. Plant Indus. Off. Cotton, Truck, Forage Crop Disease Inves. News Notes, v. 7, no. 22, p. 5. 1918. Mimeographed.

standards of the bacteriologist can only be approximated. The strain of the fungus used in the experimental work to be discussed was obtained from Kentucky Wonder beans in a garden at Blacksburg in the fall of 1917. In an effort to obtain a single spore strain, bean plants were inoculated sparingly with urediniospores, and only a few well-isolated sori were obtained. Urediniospores from a single sorus were used again for a sparing inoculation on other plants. The repetition of this procedure through several spore generations provided a strain which could, with reasonable certainty, be considered to have arisen from a single urediniospore. Stock cultures of this strain were maintained on plants of Tennessee Green Pod, and all of the inoculum used was taken from this variety.

The method of inoculation used throughout the work consisted in the atomization of the host with a urediniospore suspension followed by incubation in a moist chamber for 24 hours. One of the authors (*3*) in studies with the crownrust of oats had found that the best infection was obtained when dry spores from a culture of rusted oat seedlings were allowed to fall on plants which had been previously atomized with water. Melhus and Durrell (*8*) have also found dusting the spores on the moistened plants preferable to the use of spore suspensions. They have found a considerable reduction in the percentage of germination of urediniospores of this rust after passing them through an atomizer. There seems, however, to be greater probability of securing uniformity of dosage with spore suspensions, and this method has, therefore, been followed in our work.

A standard dosage was used throughout the experiments, the inoculum for each plant being the spores from one mature uredinium, grown on Tennessee Green Pod, applied in 1 cc. of water. In practice 40 plants were inoculated at one time, the dosage being the urediniospores from 40 sori in 40 cc. of water.

Since it had been determined previously that the susceptibility of the bean leaf varies somewhat according to the degree of maturity, the inoculations were made with the plants in as near the same stage of development as possible, the rule followed being to select the time when the first trifoliate leaf had reached a length of about ½ inch. At this time the primordial leaves have attained practically their full size but are still young and in a susceptible state. The number of days required to reach this stage of development from the planting of the seed varies with the conditions in the greenhouse between 11 and 20 days, averaging about 14 days.

In order to secure uniformity of exposure to infection an inoculation frame is employed to hold 40 four-inch pots each of which contains a single bean plant. Four varieties are inoculated in each test, 10 plants to a variety, the varieties being distributed through the frame in such a

manner as to overcome any inequality in inoculation. Plate 69, A, shows the frame filled in preparation for inoculation. The inoculum, as stated previously, is applied with an atomizer, all plants receiving as nearly as possible the same amount of material. A very fair degree of uniformity in the resultant infection is obtained as a rule by this method throughout the 10 plants of a variety. The results of one test are shown in Table I.

TABLE I.—*Experiment No. 29. Results of inoculation of four varieties of beans planted December 9 and inoculated December 31 at the rate of one sorus per plant; sori visible January 8, sporulating January 10; count made January 17*

Plant No.	Tennessee Green Pod.						McCaslan.					
	Leaf No. 1.			Leaf No. 2.			Leaf No. 1.			Leaf No. 2.		
	Number of sori.	Number of flecks.	Area.	Number of sori.	Number of flecks.	Area.	Number of sori.	Number of flecks.	Area.	Number of sori.	Number of flecks.	Area.
			Square inches.			*Square inches.*			*Square inches.*			*Square inches.*
1	206	.....	8. 73	213	.....	8. 47	452	.....	10. 64	194	.....	9. 37
2	134	.....	5. 32	86	.....	5. 19	310	.....	8. 53	319	.....	7. 90
3	180	.....	6. 33	90	.....	6. 14	225	.....	10. 60	281	.....	9. 52
4	299	.....	6. 00	269	.....	5. 17	205	.....	5. 23	221	.....	5. 72
5	132	.....	5. 98	209	.....	5. 47	142	.....	5. 35	163	.....	4. 95
6	337	.....	6. 23	156	.....	6. 03	400	.....	6. 60	191	.....	5. 97
7	113	.....	5. 30	247	.....	5. 96	286	.....	6. 98	257	.....	7. 03
8	245	.....	6. 10	240	.....	6. 63	278	.....	6. 00	302	.....	6. 40
9	102	.....	3. 98	178	.....	4. 55	356	.....	5. 40	352	.....	5. 60
10	132	.....	4. 47	144	.....	4. 29	263	.....	6. 80	134	.....	6. 20
Total	1, 880	.....	58. 44	1, 832	.....	57. 90	2, 917	.....	72. 13	2, 414	.....	68. 66

Plant No.	Improved Goddard.						Dwarf Black.					
	Leaf No. 1.			Leaf No. 2.			Leaf No. 1.			Leaf No. 2.		
	Number of sori.	Number of flecks.	Area.	Number of sori.	Number of flecks.	Area.	Number of sori.	Number of flecks.	Area.	Number of sori.	Number of flecks.	Area.
			Square inches.			*Square inches.*			*Square inches.*			*Square inches.*
1	.....	196	10. 90	.....	158	11. 80	44	42	4. 57	26	25	4. 40
2	.....	140	10. 78	.....	78	12. 00	47	39	7. 70	36	30	7. 69
3	.....	76	8. 78	.....	82	8. 97	47	39	4. 50	47	19	5. 69
4	.....	106	9. 45	.....	53	10. 40	50	17	3. 75	17	9	3. 90
5	.....	148	13. 79	.....	137	14. 13	9	3	5. 68	31	12	4. 43
6	.....	118	11. 55	.....	179	12. 15	30	28	4. 15	10	4	4. 60
7	.....	136	11. 37	.....	93	10. 55	10	10	4. 93	14	16	3. 94
8	.....	71	13. 85	.....	179	13. 90	31	20	7. 10	65	22	6. 75
9	.....	91	9. 86	.....	92	9. 70	49	31	5. 80	41	34	6. 35
10	.....	139	15. 50	.....	139	15. 54	36	32	5. 07	28	20	5. 04
Total	.....	1, 221	115. 83	.....	1, 190	119. 14	353	261	53. 25	315	191	52. 79

TABLE II.—*Summary of experiment No. 29. Percentage of total infection and fertile infection in terms of Tennessee Green Pod, which is taken as the standard and rated 100 per cent*

	Tennessee Green Pod.	McCaslan.	Improved Goddard.	Dwarf Black.
Total number of sori on 20 leaves	3,712	5,331	.....	668
Total number of flecks on 20 leaves	.....	.....	2,411	452
Total number of infections on 20 leaves	3,712	5,331	2,411	1,120
Total area in square inches	116.34	140.79	234.97	106.04
Number of sori per square inch	31.91	37.87	.....	6.30
Number of flecks per square inch	.....	.....	10.26	4.26
Number of infections per square inch	31.91	37.87	10.26	10.56
Percentage of total infection	100.00	118.68	32.15	33.09
Percentage of fertile infection	100.00	118.68	.....	19.74
Percentage of abortive infection [1]	.....	.....	100.00	40.36

[1] Calculated from total infection on each variety separately.

Following inoculation the plants are removed to a moist chamber large enough to hold all 40 plants. They are kept here in a humid atmosphere for 24 hours and are then set on the greenhouse bench. The incubation period varied within limits of 8 to 14 days during the tests, the average time from inoculation to the rupture of the sorus being 10 days. The sori are visible two or more days prior to sporulation. The final data on infection are secured 7 days after sporulation begins. This permits a full development of the initial infection on all varieties but comes early enough to exclude secondary sori which develop around the primary sorus in the more susceptible varieties (Pl. 71, B). At this time the primordial leaves are removed from the plants and the number of infections (both sori and flecks) are determined. The typical infection obtained on susceptible varieties is shown on a leaf of Dutch Case Knife in Plate 70, A, and abortive infection or flecking on a leaf of Improved Goddard in Plate 70, B.

A photographic retouching frame has been found a great aid in the counting of infections. The leaves are placed on a glass plate in this frame before an incandescent lamp, and by this lighting it is possible to determine the number of infections on both leaf surfaces at the same time, thus preventing the duplication which would result from separate counts. Infection takes place with equal readiness on both upper and lower surfaces, but with the method of inoculation used the greater number develop on the upper surface. In many cases the sorus breaks through on both surfaces. Before the count is taken the leaf surfaces are brushed with a camel's-hair brush to remove dirt and superfluous spore powder. Following the count the area of the leaf is determined by means of a planimeter, and the number of infections is reduced to a unit area basis in all varieties.

VARIATION IN DEVELOPMENT OF INFECTION

The normal development on the more susceptible varieties, of which Tennessee Green Pod is an example, is described as follows from inoculation made on January 20, 1919. The inoculation was made at 5 p. m., and after 24 hours in the moist chamber the plants were placed on the greenhouse bench. The first evidence of infection was noted 136 hours after inoculation. The sori at this time were very faint indeed, being scarcely visible. In 143 hours they were evident on close examination as minute pale spots. They were quite distinct after 161 hours, had taken on a pale yellow color, and were slightly elevated above the leaf surface. At 183 hours the centers of the sori were orange-red in color, showing sporulation, and were conspiciously elevated. The epidermis had ruptured on some of the larger sori in 190 hours, and the uredinio-spores were exposed. A ring of secondary sori develops around the primary sorus on the more susceptible varieties.

Variations from the normal development are found in a number of varieties. These variations may be listed as follows: (a) production of flecks, (b) production of telia instead of uredinia, or sori containing both uredinio-spores and teliospores, (c) production of uredinia subnormal in size, (d) lengthening of incubation period.

The term fleck is used to indicate an abortive infection. The flecks produced on different varieties vary somewhat in shape and size (Pl. 73, A, B). The flecks which develop on Tennessee Wonder (Pl. 73, C) are seen on both leaf surfaces, but are most conspicious on the lower surface. They are circular in outline, with a slightly irregular border. The spot is concave, the tissue being noticeably shrunken. A definite bronze-brown rim bounds the fleck. This is narrow in some flecks and may extend well toward the center in others. An island of green tissue may remain in the center, and this may protrude slightly, suggesting the beginning of a sorus. In some varieties a small sorus is developed in the center of some of the flecks (Pl. 69, B, *e*, *f*). The flecks on any variety are usually larger than the sori, their average diameter being from two to three times that of the sori.

The development of flecks is characteristic of certain varieties and is considered an indication of resistance. No flecks have ever been seen on Tennessee Green Pod or other very susceptible varieties, but they are regularly produced on certain others, notably Tennessee Wonder, Improved Goddard, and Hodson Green Pod. On some varieties all the infections develop into flecks, while on others there may be a considerable number of normal sori developed and only a few flecks. The percentage of flecking with the different varieties is shown in Table III.

TABLE III.—*Susceptibility of different varieties of beans to rust infection, with infection on Tennessee Green Pod as standard and rated 100 per cent*

BUSH BEANS, GREEN POD

Experiment No.	Variety.	Average leaf area.	Number of infections per square inch.		Percentage of infection.		
			Flecks.	Sori.	Total.	Abortive.	Fertile.
		Square Inches.					
12a	Pink	4.52	.....	9.82	119.61	.....	119.61
27	Snowflake	2.67	.....	38.86	116.31	.....	116.31
27	Navy Pea	2.51	.....	36.92	110.50	.....	110.50
10	Tepary	2.28	.....	7.79	101.43	.....	101.43
5	Pinto	4.43	.....	65.88	89.20	.....	89.20
24	Lady Washington	4.05	1.87	28.76	79.10	6.12	73.83
8	Red Valentine	4.42	.....	55.09	57.29	.....	57.29
31	Blue Pod Navy	3.65	.....	05	43.60	.....	43.60
7	Red Kidney (Wells)	10.58	.....	45.76	39.39	.....	39.39
4	Emerald Beauty	10.47	.56	10.55	38.52	5.04	36.58
20	White Kidney	8.41	.....	11.34	33.35	.....	33.35
21	Burpee's Stringless	6.69	8.10	12.62	53.01	39.05	32.28
7	Full Measure	12.29	.....	37.19	32.01	.....	32.01
15	White Marrow	6.23	.....	2.92	25.15	.....	25.15
19	Warren	11.86	3.16	4.91	32.77	39.19	19.94
9	Horticultural, Dwarf	7.34	7.47	41.37	22.40	15.29	18.97
19	Round Six Weeks	6.86	.....	3.58	14.54	.....	14.54
14	French's Horticultural	8.98	1.15	2.96	16.36	27.91	11.78
18	Bountiful	8.24	1.00	1.69	13.96	36.68	8.77
34	Bird Eye	6.31	.....	.86	8.62	.....	8.62
32	Yellow Eye	9.54	.....	.69	6.97	.....	6.97
10	Longfellow	8.65	.....	.50	6.50	.....	6.50
24	Giant Stringless	7.69	.....	1.94	4.98	.....	4.98
8	Black Valentine	4.88	19.59	1.15	21.57	94.47	1.19
12a	Mexican Red	6.79	.16	.02	2.19	87.50	.24
24	Early Refugee	7.07	.84	.02	2.21	97.52	.05
14	Low's Champion	6.68	1.68	.01	6.73	99.51	.04
29	Improved Goddard	11.75	10.26	.....	32.15	100.00	.....
33	May Queen	6.25	1.97	.....	10.83	100.00	.....
18	Early Mohawk	11.86	1.95	.....	10.12	100.00	.....
18	Refugee	5.99	1.51	.....	7.83	100.00	.....
30	Hodson Green Pod	12.13	1.14	.....	5.81	100.00	.....

BUSH BEANS, WAX POD

2	Keeney's Rustless	6.97	6.51	12.03	38.89	35.15	25.24
29	Dwarf Black	5.30	4.26	6.30	33.09	40.36	19.74
33	Scarlet Wax	10.13	.66	2.23	15.88	23.11	12.25
2	Currie	8.22	18.78	1.84	43.26	91.09	3.17
23	California	6.38	10.18	.....	63.46	100.00	.....
22	Webber	7.06	12.47	.....	50.71	100.00	.....
22	Flagolet	7.28	12.18	.....	49.53	100.00	.....
26	Crystal White	3.10	5.19	.....	44.24	100.00	.....
23	Challenge	4.91	4.80	.....	29.93	100.00	.....
22	Wardwell	6.65	6.58	.....	26.76	100.00	.....
23	New Pearl	7.56	3.22	.....	20.07	100.00	.....
27	Hodson Wax	6.68	5.88	.....	17.60	100.00	.....
23	Detroit	8.57	1.85	.....	11.53	100.00	.....
26	Golden Eye	8.31	1.08	.....	9.21	100.00	.....

TABLE III. *Susceptibility of different varieties of beans to rust infection, with infection on Tennessee Green Pod as standard and rated 100 per cent*—Continued

POLE BEANS, GREEN POD

Experiment No.	Variety.	Average leaf area.	Number of infections per square inch.		Percentage of infection.		
			Flecks.	Sori.	Total.	Abortive.	Fertile.
		Square Inches.					
29	McCaslan	7.04	.....	37.87	118.68	.....	118.68
20	Virginia Cornfield	4.11	.....	36.07	106.09	.....	106.09
32	Cut Short	5.30	.....	3.19	104.61	.....	104.61
11	Kentucky Wonder	7.46	.....	5.75	80.87	.....	80.87
28	Dutch Case Knife	5.89	.....	36.85	77.50	.....	77.50
9	Royal Corn	5.03	.....	146.04	66.97	.....	66.97
26	Powell's Prolific	5.90	.....	6.82	58.14	.....	58.14
28	Creaseback	4.03	.....	24.32	51.12	.....	51.12
1	Lazy Wife	7.21	.....	2.68	13.74	.....	13.74
1	Horticultural, Pole	9.78	.....	2.12	10.87	.....	10.87
13a	Brockton	10.95	0.21	.05	6.77	80.70	1.31
7	Tennessee Wonder	10.09	63.10	.68	54.91	98.93	.59
13a	Marblehead	8.76	.10	.01	2.86	90.00	.26

POLE BEANS, WAX POD

17	Golden Cluster	5.84	.....	4.80	71.22	.....	71.22
28	Kentucky Wonder Wax	5.73	.....	32.32	67.94	.....	67.94
21	Mont d'Or	9.60	3.81	.....	9.75	100.00	.....
20	Everbearing	9.80	.45	.....	1.32	100.00	.....
17	Indian Chief	10.52	.....	.....	.....	.....	.....

In general the flecks are visible some time before the sori are evident. Accompanying the inoculation of Tennessee Green Pod on January 20, described previously, in which 143 hours elapsed before the "anlage" of the sori were plainly visible, flecks were visible on Hodson Green Pod in 112 hours and on Improved Goddard and Tennessee Wonder in 123 hours.

On certain varieties the immediate results of the inoculation with urediniospores is seen in the production of telia or of mixed sori. This is particularly true of Black Valentine and of some of the wax-podded varieties, notably Challenge, Flagolet, Golden Eye, Pencil Pod, Keeney, Wardwell, and New Kidney.

The size of uredinia will vary to some extent on a given variety according to the degree of infection and crowding. The average size of the sorus on different varieties varies greatly, however, ranging from about 180 μ on Dwarf Black Wax to 600 μ on Tennessee Green Pod (compare A and B, Pl. 72, and *a* and *d*, Plate 69, B).

The duration of the incubation period is modified by environment, especially temperature, but in different varieties under similar conditions a variation of several days may occur. Such variations have

been noted repeatedly. In order to have more definite data on this point two plants each of 72 varieties were inoculated on March 29, 1920, and were held under similar conditions. Thirty-seven of the varieties produced uredinia only, 3 both uredinia and flecks, 27 flecks only, and 5 neither sori nor flecks. The number of days elapsing before the uredinial "anlage" were visible varied from 6 to 10 on different varieties, and the time of sporulation from 10 to 15 days. The "anlage" were visible on 6 varieties on the sixth day, on 9 varieties on the seventh day, on 22 varieties on the eighth day, on 1 on the ninth day, and on 2 on the tenth day. Sporulation ensued on 6 varieties on the tenth day, on 30 on the eleventh day, on 2 on the twelfth day, on 1 on the fourteenth, and on 1 on the fifteenth. The number of days between the appearance of the "anlage" and sporulation varied from two to five, the mean being three days. As a general rule the sorus develops more rapidly on the more susceptible varieties than on the more resistant. The flecks on the resistant varieties were visible, as a rule, sooner than the "anlage" on the susceptible varieties. Flecks were observed on 9 varieties on the fifth day, on 16 on the sixth day, on 4 on the seventh, and on 1 on the eighth.

RELATIVE SUSCEPTIBILITY OF BEAN VARIETIES

In our previous publication (*4*) a number of varieties of beans were classified according to their susceptibility to rust under field conditions. Four classes were recognized—rust-free, rust-proof, rust-enduring, and rust-susceptible. In this work we have attempted to obtain a more definite expression of susceptibility by using a susceptible variety as a standard and expressing the relative susceptibility of other varieties in terms of that standard. A standard dosage is employed and the relative susceptibility of a variety is indicated by the percentage of infection obtained, modified by the amount of flecking, the size of the sorus, and the length of the incubation period.

The determination of the degree of infection is based on the assumption that all varieties receive approximately the same number of urediniospores per unit area of leaf surface exposed as the standard. The resultant infection is then determined by count and is calculated as average number of infections per square inch. The number of sori obtained on Tennessee Green Pod is then rated 100 per cent, and the number of infections on the other varieties in the test is reduced to percentage of the standard. The percentage of infection is expressed as total infection (both sori and flecks), fertile infection (sori only), and abortive infection (flecks). Total infection and fertile infection are in terms of the standard, but abortive infection relates only to the variety in question and is the percentage of the total infections aborted on that variety. The details of this method are illustrated in Tables I and II. Table III is a summary of the experiments and shows the degree of infection obtained on the 64 varieties tested.

The varieties are grouped for convenience according to the dwarf or running habit and the color of the pod. Of the 46 varieties of dwarf or bush beans, 32 have green pods and 14 wax pods. Of the 18 varieties of pole beans, 13 have green pods and 5 wax pods.

The very marked variation in the susceptibility of the different varieties under the conditions of the experiments is evident. They are arranged in the different groups according to susceptibility to infection, the production of sori (percentage of fertile infection) being used as the basis for comparison in those varieties which produce sori rather than the percentage of total infection.

In Table IV the 64 varieties are grouped under the four classes, and the comparative susceptibility of the classes as a whole is shown. It is apparent that pole beans with green pods constitute the most susceptible class and bush beans with wax pods the least susceptible, that pole beans are more susceptible as a class than bush beans, and that green-pod varieties are more susceptible than wax-pod varieties.

Considering that the production of sori is an indication of greater susceptibility than the production of flecks, it is seen that 67 per cent (12 in 18) of the pole varieties produced sori unaccompanied by flecks as compared with 35 per cent (16 in 46) of the bush beans, while only 11 per cent of the former class produced flecks unaccompanied by sori as compared with 33 per cent of the latter class. Comparing green-pod varieties with wax-pod varieties, the percentage with sori only is 58 as against 11, and for flecks only the percentage is 11 as against 63. Comparing severity of infection on individual varieties, it is seen that the fertile infection was 50 per cent or more of the standard in 55 per cent of the pole varieties as against 15 per cent of the bush varieties, and in 33 per cent of the green pods as against 11 per cent of the wax pods.

TABLE IV.—*Susceptibility of different classes of bean varieties to rust*

	Bush.		Pole.	
	Green.	Wax.	Green.	Wax.
Number of varieties tested	32	14	13	5
Number bearing sori only	16	.....	10	2
Number bearing sori and flecks	11	4	3	.....
Number bearing flecks only	5	10	.....	2
Number showing no infection	.....	.....	.....	1
Number showing fertile infection 50 per cent or more of standard	7	.....	8	2

CORRELATION BETWEEN SEED CHARACTERS AND SUSCEPTIBILITY TO RUST

In order to determine whether susceptibility to rust is associated with any particular color pattern or shape of the seed, a study of the seed

characters of a number of varieties was made. The data on seed characters have been drawn largely from Jarvis (*6*), supplemented by observations when necessary. The details of this study are shown in Table V. Only two classes with respect to susceptibility are provided, the rust-enduring, rust-proof, and rust-free varieties all being classed as resistant. Although it is probably impossible to draw conclusions that would not be subject to exceptions, nevertheless the following general statements can be made for the 51 varieties included in the study. With respect to color, all solid red or red mottled beans are rust-resistant. With respect to shape, all marrow beans are rust-resistant. White beans seem to be more susceptible as a group than black or brown beans, and pea beans more so than medium or kidney.

VARIABILITY OF VARIETIES

It is of interest to note to what extent the individual plants of a variety differ in behavior to rust infection. Although the number of plants of a variety under observation in the experiments is not large enough to justify more than tentative conclusions, sufficient additional evidence from field trials is available to indicate that the stability or variability of a variety as indicated below is approximately correct. The majority of varieties studied were stable with respect to rust infection, all individuals responding in the same way. The standard used, Tennessee Green Pod, is especially reliable in this respect. Of the 340 plants inoculated in the experiments all developed sori, and not a single fleck was observed. The stable varieties are grouped as follows, according to the character of the response to inoculation.

TABLE V.—*Correlation between seed characters and susceptibility to rust*[1]

Color of seed.	Pea.	Medium.	Marrow.	Kidney.	Totals.
White	2/7	1/2	2/2	1/5	6/16
White, yellow eye	.....	.....	2/2	.....	2/2
Black	.....	2/3	1/1	.....	3/4
Brown	.....	1/1	2/2	3/5	6/8
Yellow, brown ring	.....	1/1	.....	1/1	2/2
Red	.....	.....	4/4	1/1	5/5
Red on fawn or buff ground	.....	.....	3/3	5/5	8/8
Pink	.....	0/1	.....	.....	0/1
Brown, purple, or black on fawn or buff ground.	.....	0/1	.....	4/4	4/5
Total	2/7	5/9	14/14	15/21	36/51

[1] The denominator shows the total number of varieties, the numerator the number that are rust-resistant.

ALL PLANTS PRODUCING SORI ONLY, NO FLECKS.—Pink, Snowflake, Navy Pea, Tepary, Pinto, Red Kidney, White Kidney, Full Measure, Round Six Weeks, Longfellow, Giant Stringless, McCaslan, Virginia Cornfield, Cut Short, Kentucky Wonder, Dutch Case Knife, Royal Corn, Powell's Prolific, Creaseback, Golden Cluster, and Kentucky Wonder Wax.

ALL PLANTS PRODUCING FLECKS ONLY, NO SORI.—Refugee, Early Mohawk, May Queen, Improved Goddard, California, Webber, Flagolet, Challenge, Wardwell, New Pearl, Hodson Wax, Detroit, Golden Eye, and Mont d'Or.

ALL PLANTS PRODUCING BOTH SORI AND FLECKS, THE SORI BORNE EITHER WITHIN THE FLECKS OR INTERMIXED WITH THEM.—Dwarf Black Wax, Burpee's Stringless, Tennessee Wonder, and Black Valentine.

ALL PLANTS CLEAN, PRODUCING NEITHER SORI NOR FLECKS.—Indian Chief.

The individual plants of the remaining 25 varieties were somewhat variable in response to rust inoculation. This is shown in Table VI. In the varieties Blue Pod Navy, Yellow Eye, Lady Washington, White Marrow, and Bird Eye, a large percentage of the individuals developed sori and a small percentage showed no infection, while these conditions were reversed in Lazy Wife. There were no intermediate or flecking plants in any of these.

TABLE VI.—*Response of individual plants of unstable varieties to rust inoculation*

Variety.	Percentage of plants showing—			
	Sori only.	Sori and flecks.	Flecks only.	Clean.
Blue Pod Navy	95	.....	.....	5
Yellow Eye	95	.....	.....	5
Emerald Beauty	90	10	.....	.....
Lady Washington	90	.....	.....	10
White Marrow	90	.....	.....	10
Bird Eye	90	.....	.....	10
May Queen	60	40	.....	.....
Horticultural Pole	55	5	5	35
Bountiful	50	25	25	.....
Horticultural Dwarf	45	.....	5	50
Red Valentine	40	55	.....	5
Warren	35	10	55	.....
French's Horticultural	30	70	.....	.....
Keeney	30	70	.....	.....
Brockton	20	.....	50	30
Currie	10	65	25	.....
Early Refugee	10	10	60	20
Marblehead	10	10	40	40
Mexican Red	10	.....	50	40
Lazy Wife	5	.....	.....	95
Scarlet Wax	.....	90	10	.....
Low's Champion	.....	40	60	.....
Hodson Green Pod	.....	5	95	.....
Everbearing	.....	.....	20	80

The greatest variability is shown in the varieties Horticultural Pole, Horticultural Dwarf, Brockton, Early Refugee, Marblehead, and Mexican Red. All had a considerable percentage of clean plants, some that developed sori, and some intermediates.

SIZE OF THE SORUS AS AN INDEX OF SUSCEPTIBILITY

In addition to the degree of infection and the production of flecks, the size of the sorus produced on different varieties also provides an index of relative susceptibility. As noted previously, great variation exists in the size of the sori produced on different varieties. The size of the sorus in turn is an index to the spore production of the sorus, and any marked curtailment in the number of spores produced may readily be conceived to be a limiting factor of considerable importance in the propagation of the pathogene. The production of a small sorus is evidently to be considered in the same light as the production of flecks—as a response to an unfavorable medium.

The average size of the uredinium on each variety has been determined by measurement of a number of uredinia. The relative spore-producing capacity is then determined by calculating the number of urediniospores produced in a layer one spore in thickness covering the sorus. This does not represent the total spore-producing capacity of the sorus but gives a basis for comparison between varieties. The main objection to this expression is that it does not magnify the differences between the more susceptible and more resistant varieties sufficiently. It seems evident from examination that the more susceptible varieties produce more layers of spores in a uredinium than the more resistant ones. No differences have been found in the size of the urediniospores borne on different varieties.

In determining the number of spores produced by a sorus, the number in the diameter is determined by calculation. The urediniospore is approximately 20 μ in diameter, and 30 such spores would be contained in the diameter of a sorus 600 μ broad. The square of the number in the diameter multiplied by 0.7854 gives the number in one layer covering the sorus. The sorus on Tennessee Green Pod, which is one of the largest, averages 600 μ in diameter and, therefore, has a spore-producing capacity of 706.8 spores in one layer, while that on Bird Eye, averaging 300 μ, has a spore-producing capacity of only 176.7. The spore-producing capacity of the sorus on different varieties has been reduced to percentages for purposes of comparison. The sorus on Tennessee Green Pod is again used as the standard, and its spore-producing capacity is rated 100 per cent. The varieties on which data were obtained are grouped in Table VII according to spore production.

It will be noted in a comparison between Tables VII and III that varieties with high rating in percentage of fertile infection also show rate high in spore-producing capacity. Tepary is the only variety rating above 50

per cent in fertile infection which falls below 50 per cent in spore-producing capacity, while three varieties, White Marrow, Full Measure, and Keeney's Rustless, rating below 50 per cent in fertile infection, have spore-producing capacities in excess of 50 per cent. In general it can be stated that the production of large sori is an indication of greater susceptibility to infection than the production of small sori.

TABLE VII.—*Relative spore-producing capacity of the uredinia on different varieties*

Spore-producing capacity.		Variety.
Per cent.	Number.	
100	707	Tennessee Green Pod, Navy Pea, Snowflake, Lady Washington, McCaslan, Virginia Cornfield, Dutch Case Knife, Powell's Prolific, Creaseback, Cut Short, Golden Cluster.
70	491	Pinto, White Marrow, Kentucky Wonder, Royal Corn.
54	380	Pink, Red Valentine, Full Measure, Keeney's Rustless.
44	314	Tepary, Blue Pod Navy, Red Kidney, White Kidney, Horticultural Dwarf, Round Six Weeks, Longfellow, Low's Champion, Brockton, Tennessee Wonder, Kentucky Wonder Wax.
32	227	Early Refugee, Giant Stringless, Bountiful, Horticultural Pole.
25	177	Warren, Bird Eye, French's Horticultural, Black Valentine, Marblehead, Currie.
16	113	Yellow Eye, Burpee's Stringless, Mighty Nice, Mexican Red.
11	79	Scarlet Wax, Crystal White, Dwarf Black.

SUSCEPTIBILITY OF BEAN VARIETIES UNDER GREENHOUSE CONDITIONS AS COMPARED WITH FIELD CONDITIONS

The question is naturally raised in connection with greenhouse experiments as to what extent they serve as a reliable index of field behavior. All the varieties of beans used in our greenhouse work have been grown in the field, most of them during two seasons, where they have been subjected to uniform exposure to rust infection by inoculation. The results of the first season's field tests have been described in a previous paper (*4*). In the 1918 tests the varieties were classified according to relative susceptibility in four groups, as follows: Susceptible, rust-enduring, rust-proof, rust-free. Yields were obtained only for the dwarf varieties commonly grown for dry-shell purposes and from the pole beans. In the 1919 tests some of the most susceptible varieties were eliminated and some additional varieties were added. This season was particularly favorable for the development of anthracnose, and some of the varieties suffered severe losses from this disease and to a lesser degree from bacterial blight. With respect to rust infection, the varieties respond in the same way during the two seasons, but the effects

of the rust attack were not so noticeable in 1919 as in 1918. The susceptible varieties were severely rusted, but defoliation was not so severe until later in the season, and their yield was better in comparison with that of the more resistant varieties than in 1918. No data on the curtailment of the yield of the pole varieties as determined by rust infection could be obtained because of the severity of anthracnose on certain varieties.

The greenhouse and field behavior of the dry-shell varieties is compared in Table VIII, and that of the pole varieties in Table IX. The varieties are listed according to susceptibility in the greenhouse.

Of the 17 varieties in Table VIII the first 7, which were classed as susceptible in the field, all rated above 70 per cent fertile infection in the greenhouse, while the 9 varieties in the resistant classes—enduring, proof, and free—all fell below 50 per cent fertile infection. Robust, not included in the greenhouse tests, was classed as rust-proof in the field and outyielded all other varieties in 1919. This is the only small white bean in our tests, with the exception of Blue Pod Navy, which has successfully withstood the attack of rust. Robust seems to be an especially desirable variety in that it combines resistance to mosaic, as shown by Reddick and Stewart (*9*), with rust resistance, attractive appearance, and desirable commercial type. Bird Eye, grown for the first time in 1919, was classed as rust-free and was second to Robust in yield. This variety is similar to Yellow Eye in seed characters and is popular in some localities in Virginia. It is attractive and of good quality and seems to be very dependable. The yields in 1918 are more representative of the curtailment of production by rust than those in 1919, since anthracnose was severe in the latter year. The yields of Improved Goddard, Horticultural Dwarf, and White Kidney were reduced materially by anthracnose. Bird Eye and Well's Red Kidney were especially free from anthracnose. Robust was quite free from anthracnose in 1919 but was severely injured in 1920.

Of the 18 varieties of pole beans listed in Table IX the 8 which were classed as susceptible all exceeded 50 per cent fertile infection in the greenhouse. All the rust-proof and rust-free varieties fell below 50 per cent; but the two rust-enduring varieties, Golden Cluster and Kentucky Wonder Wax, rated 71 and 68 per cent. The yield of all of the susceptible varieties was severely curtailed by the rust attack in 1918, 5 of them being complete failures (Pl. 71, A). McCaslan was not grown in 1918 but was seriously damaged in 1919. No yield data for 1919 are given, as explained previously, because of the severity of anthracnose. This injury was especially severe on Indian Chief, Everbearing, Mont d'Or, Marblehead, Brockton, and Lazy Wife.

TABLE VIII.—*Comparison between greenhouse and field behavior of dry-shell beans*

Variety.	Percentage of fertile infection in greenhouse.	Field.			
		Susceptibility to rust.	Yield of dry beans.		Severity of anthracnose.
			1918.	1919.	
			Gm.	*Gm.*	
Pink	120	Susceptible	[a] 14	.....	
Snowflake	116	do	[a] 386	.....	
Navy Pea	111	do	[a] 313	[a] 2,390	Slight.
Tepary	101	do	[a] 0	.....	
Tennessee Green Pod	100	do	[a] 270	.....	
Pinto	89	do	[a] 153	.....	
Lady Washington	74	do	[a] 633	[a] 2,174	Do.
Robust	[c]	Proof	.....	9,976	None.
Blue Pod Navy	44	Enduring	1,121	4,370	Moderate.
Red Kidney	39	Free	1,852	4,085	None.
White Kidney	33	do	682	[b] 2,960	Severe.
White Marrow	25	Enduring	1,119	4,024	Slight.
Horticultural Dwarf	19	Proof	1,305	[b] 2,730	Very severe.
Bird Eye	9	Free	.....	4,918	None.
Yellow Eye	7	Proof	938	3,250	Slight.
Mexican Red	−1	Free	1,090	3,185	Moderate.
Improved Goddard	−1	do	618	[b] 1,310	Very severe.

[a] Yield reduced materially by rust.
[b] Yield reduced materially by anthracnose.
[c] Not tested.

TABLE IX.—*Comparison between greenhouse and field behavior of pole beans*

Variety.	Percentage of fertile infection.	Field rating.	Yield in 1918.	Severity of anthracnose in 1919.
			Gm.	
McCaslan	119	Susceptible	.....	Slight.
Virginia Cornfield	106	do	0	
Cut Short	105	do	0	
Kentucky Wonder	81	do	109	Moderate.
Dutch Case Knife	78	do	159	Slight.
Golden Cluster	71	Enduring	263	Do.
Kentucky Wonder Wax	68	do	216	Do.
Royal Corn	67	Susceptible	0	
Powell's Prolific	58	do	0	Do.
Creaseback	51	do	0	
Lazy Wife	14	Variable	.....	Very severe.
Horticultural Pole	11	Proof	897	Slight.
Brockton	1	Free	556	Very severe.
Tennessee Wonder	−1	Proof	751	
Marblehead	−1	Free	371	Severe.
Mont d'Or	0	do	506	Very severe.
Everbearing	0	do	143	Do.
Indian Chief	0	do	261	Do.

There is in general a very satisfactory agreement between the greenhouse and field behavior of bean varieties; and the greenhouse test, properly interpreted, seems to afford a reliable basis for predicting the

relative susceptibility of a variety in the field. The greenhouse test is seemingly a more severe one than the field test. Some varieties which have developed a few sori in the greenhouse have remained entirely rust-free in the field. It seems to hold true that a variety which develops less than 50 per cent fertile infection in the greenhouse does not suffer material injury from rust in the field, and conversely that a variety which exceeds 50 per cent infection in the greenhouse may be expected to suffer material injury in the field. No exceptions to the first rule have been noted and only two apparent exceptions, Golden Cluster and Kentucky Wonder Wax, to the latter. Both these varieties were conspicuously rusted in the field, but both yielded fair crops. It is impossible to state the degree of injury sustained by these varieties, since no rust-free plants were available for comparison.

CURTAILMENT OF YIELD DUE TO BEAN RUST

Attempts have been made to obtain data on the amount of reduction in yield caused by bean rust, but the nature of the pathogene makes the securing of reliable data almost impossible. Yield data from rusted and rust-free plants should preferably be obtained from alternating rows in the same plot, but it is impossible to maintain rust-free plants of susceptible varieties in the same plot with rusted plants. It was thought that the difficulty might be overcome by growing both resistant and susceptible varieties in separate isolated rusted and rust-free plots, the resistant varieties being used as a criterion of the relative crop-producing capacities of the different soils.

A planting of this type was made in 1918 with Improved Goddard as the resistant variety and Navy Pea and Tennessee Green Pod as the susceptible varieties. The soil of the two plots was similar in physical properties and apparently so in fertility. All plants in the rusted plot were inoculated with urediniospores, and a general and severe infection resulted on the rows of susceptible varieties. The rust-free plot was not inoculated. It became infected naturally later in the season but not early enough to cause any material reduction in yield. Unfortunately the rust-resistant variety was so severely attacked by anthracnose that no reliable yield data could be obtained. The difference in growth and yield of the rust-susceptible varieties on the two plots was very marked, almost no beans being produced in the rusted plot. The yields of shelled beans for single rows 65 feet in length were as follows: Tennessee Green Pod, rust-free plot 267 gm., rusted plot 9 gm., difference 95 per cent; Navy Pea, rust-free plot 494 gm., rusted plot 95 gm., difference 81 per cent.

A similar test was made in 1920 with three susceptible and three resistant varieties. The two plots were on the same piece of land but were separated by a strip of corn. Rust was very abundant on the susceptible varieties in the inoculated plot, while the control remained free

throughout the season. The injury from rust was not so great as in 1918, apparently on account of seasonal differences; and the plants in the rusted plot retained their leaves longer and matured a fair crop. The yields are shown in Table X. The figures are in grams of shelled beans for single rows 60 feet in length and are averages of two rows for each variety. It will be noted that the three susceptible varieties all show a decrease in yield on the inoculated plot, while the three resistant varieties show an increase. It seems from this that the soil of the inoculated plot was better and that greater differences in the yield of the rusted and rust-free rows of susceptible varieties would be expected on plots of equal fertility. The three susceptible varieties show an average decrease of 28 per cent in the inoculated plot, while the three resistant varieties show an average increase of 31 per cent. It seems apparent that a reduction in yield of 50 per cent or more may be expected from a severe rust attack on susceptible varieties.

TABLE X.—*Yields of rust-susceptible and resistant varieties of beans in rusted and rust-free plots*

Variety.	Susceptibility to rust.	Rust-free plot.[1]	Rusted plot.[1]	Yield of rusted plot in percentage of rust-free plot.
Tennessee Green Pod	Susceptible	970	530	55
Pinto	do	385	217	56
Navy Pea	do	1,007	959	95
Bird Eye	Resistant	2,243	2,325	104
Yellow Eye	do	1,466	2,091	143
Red Kidney	do	2,162	3,256	151

[1] Expressed in grams per 60-foot row.

BIOLOGIC FORMS OF BEAN RUST

We have made no extended study of the occurrence of biologic forms of the bean-rust fungus, but it seems desirable to record such observations as have been made in connection with the studies on susceptibility of varieties.

The strain of rust from the kidney or garden bean which we have used in our studies has been tested on a large number of horticultural varieties of the kidney bean (*Phaseolus vulgaris*), on 9 varieties of the lima bean, (*P. lunatus* L.), 1 variety of the tepary bean (*P. acutifolius latifolius* D. F. Freeman), 18 varieties of cowpeas (*Vigna sinensis* (L.) Endl.), 1 variety of the broad or horse bean (*Vicia faba*), and 1 variety of asparagus bean (*Dolichos sesquipedalis* L.). Infection has resulted on many varieties of the kidney bean, as recorded previously, and also on the tepary bean. Slight infection only was obtained on 1 variety of lima bean in the greenhouse, but the other 8 varieties tested remained free from infection in both greenhouse and field. The infection in this case was slight and con-

sisted of small telia borne in the centers of flecks. No infection was obtained on the horse bean, the asparagus bean, nor on any variety of cowpea.

A second form distinct pathologically from the foregoing but similar morphologically was isolated in 1919 from leaves of cowpeas, variety Black Eye, which were sent us from California by Mr. D. C. Milbrath. It has been cultured in the greenhouse on plants of this variety and has been used for both greenhouse and field inoculations on a number of varieties of kidney beans and 18 varieties of cowpeas. Infection has been obtained on only the Black Eye variety of cowpea and on two similar strains, Extra Early Black Eye and Ramshorn Black Eye. No tests have been made on lima, horse, tepary, or asparagus beans.

These two strains may be designated the kidney-bean strain and the cowpea strain. They are distinct biologically, but their host ranges are as yet imperfectly known. It is possible that more than one biologic form occurs on kidney beans, but such evidence as is at hand would indicate that the strain which occurs commonly on kidney beans in the United States has the same host preferences as the one we have studied. The behavior of varieties in Maine, as observed by Morse (*9*), agrees in all essentials with our data, and the general mention of rust on Kentucky Wonder in other States points to this conclusion. It seems probable that a distinct biologic form occurs in South America. Gassner (*5*) records rust as severe on Mont d'Or and Flageolet, two-wax-pod varieties, while our strain produced flecks only on these. It is possible, however, that the varieties grown under these names by us were distinct from those used in South America. Gassner obtained his seed from Europe.

SUMMARY

A technic of inoculation and method of record is described which makes possible the expression of the relative rust susceptibility of varieties of beans in terms of percentage of a standard.

Variations from the behavior of the susceptible standard which occur in different varieties following inoculation with urediniospores in the greenhouse are of the following types: (*a*) reduction in number of infections, (*b*) reduction in the size of the uredinium, (*c*) abortion of infection or flecking, (*d*) immediate production of telia instead of uredinia, (*e*) lengthening of the incubation period. These variations are considered as responses to an unfavorable medium and as expressions of resistance.

Of the varieties studied, those with indeterminate growth (pole beans) were more susceptible as a class than those with determinate growth (bush beans). Green-pod varieties were more susceptible as a class than wax-pod varieties. With respect to color of seed all varieties with solid red or red mottled seed were rust resistant, while varieties with white seed were more susceptible as a class than those of any

other color. With respect to form of seed all varieties of the marrow type were rust resistant and those with pea beans constituted the most susceptible class.

Little or no variation in the susceptibility of individuals was found in the majority of the varieties studied, but marked variation existed between individuals in a few varieties.

Field tests which have supplemented the greenhouse tests have demonstrated that the greenhouse test affords a reliable index of the field behavior of a variety with respect to rust infection. An attack of rust on susceptible varieties in the field may result in a curtailment of production amounting to 50 per cent or more, and in very severe attacks to total loss.

The existence of two biologic forms of the bean-rust fungus has been demonstrated, but their host ranges are as yet imperfectly known.

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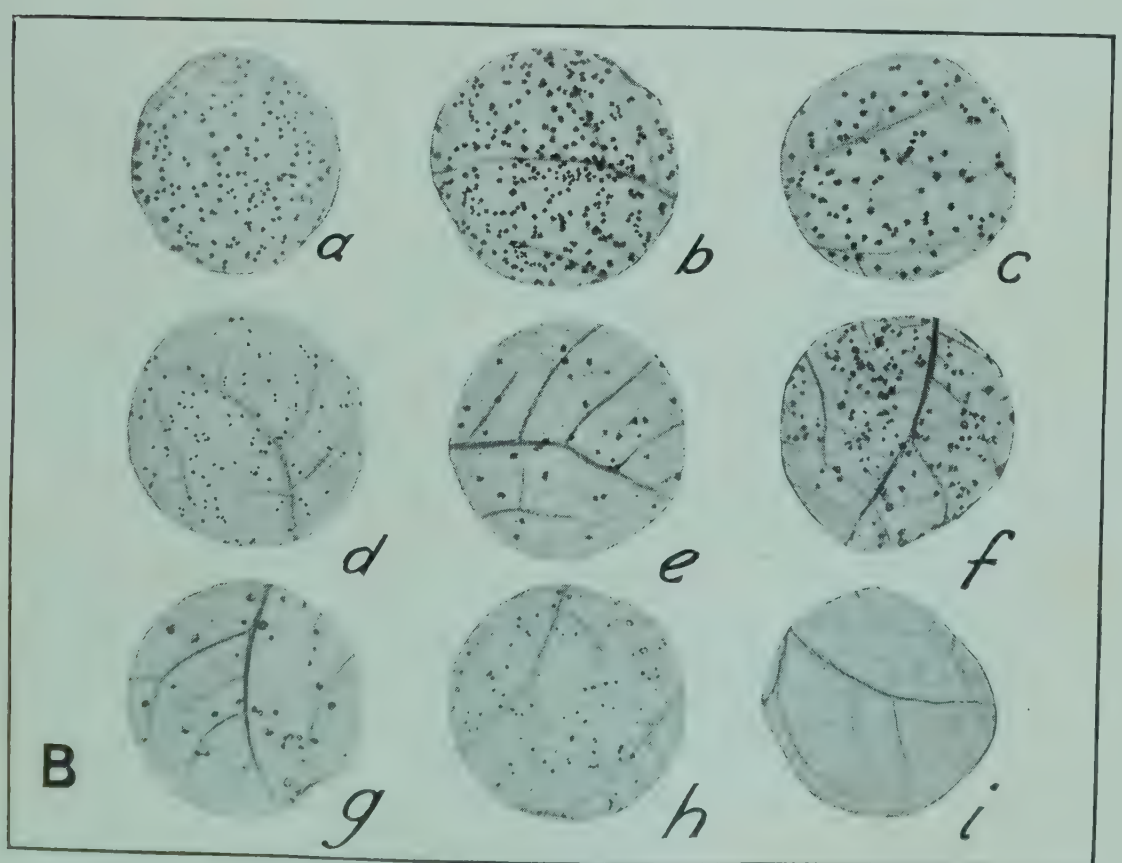
PLATE 69

A.—The frame used in the inoculation of bean plants in the greenhouse, filled with plants in position for atomization.

B.—Disks of leaves of varieties of beans photographed after extraction of chlorophyll, showing variation in response to rust inoculation in the greenhouse. All inoculated at the same time and incubated under similar conditions. *a*, Tennessee Green Pod, large uredinia; *b*, McCaslan and *c*. Navy Pea, similar to *a; d*, Yellow Eye, small uredinia; *e*, French's Horticultural, small uredinia bordered by flecks; *f*, Tennessee Wonder, small uredinia, uredinia bordered by flecks and flecks, *g*, New Pearl, large flecks; *h*, Improved Goddard, small flecks; *i*, Horticultural Pole, neither sori nor flecks.

A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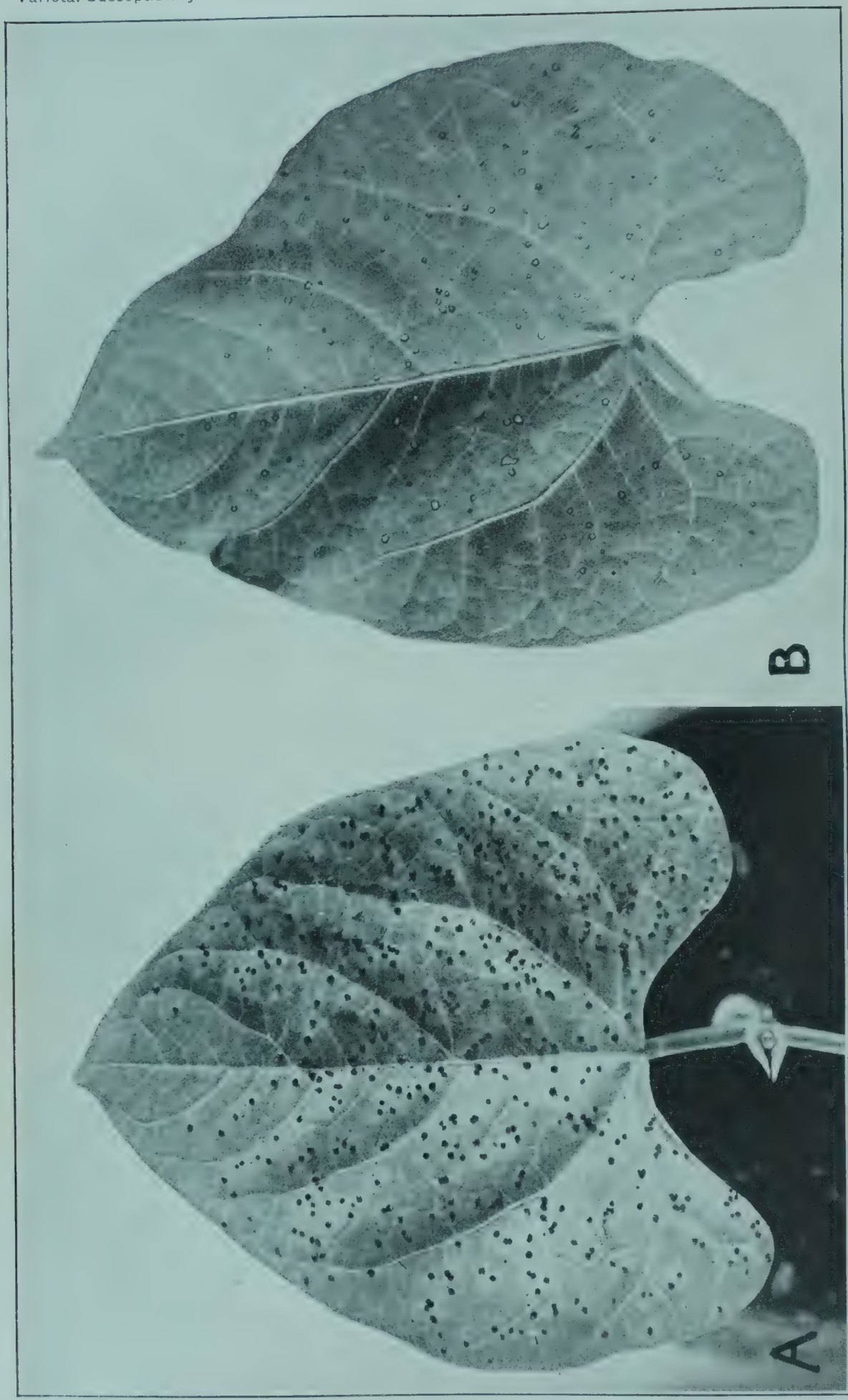

B
A

PLATE 70

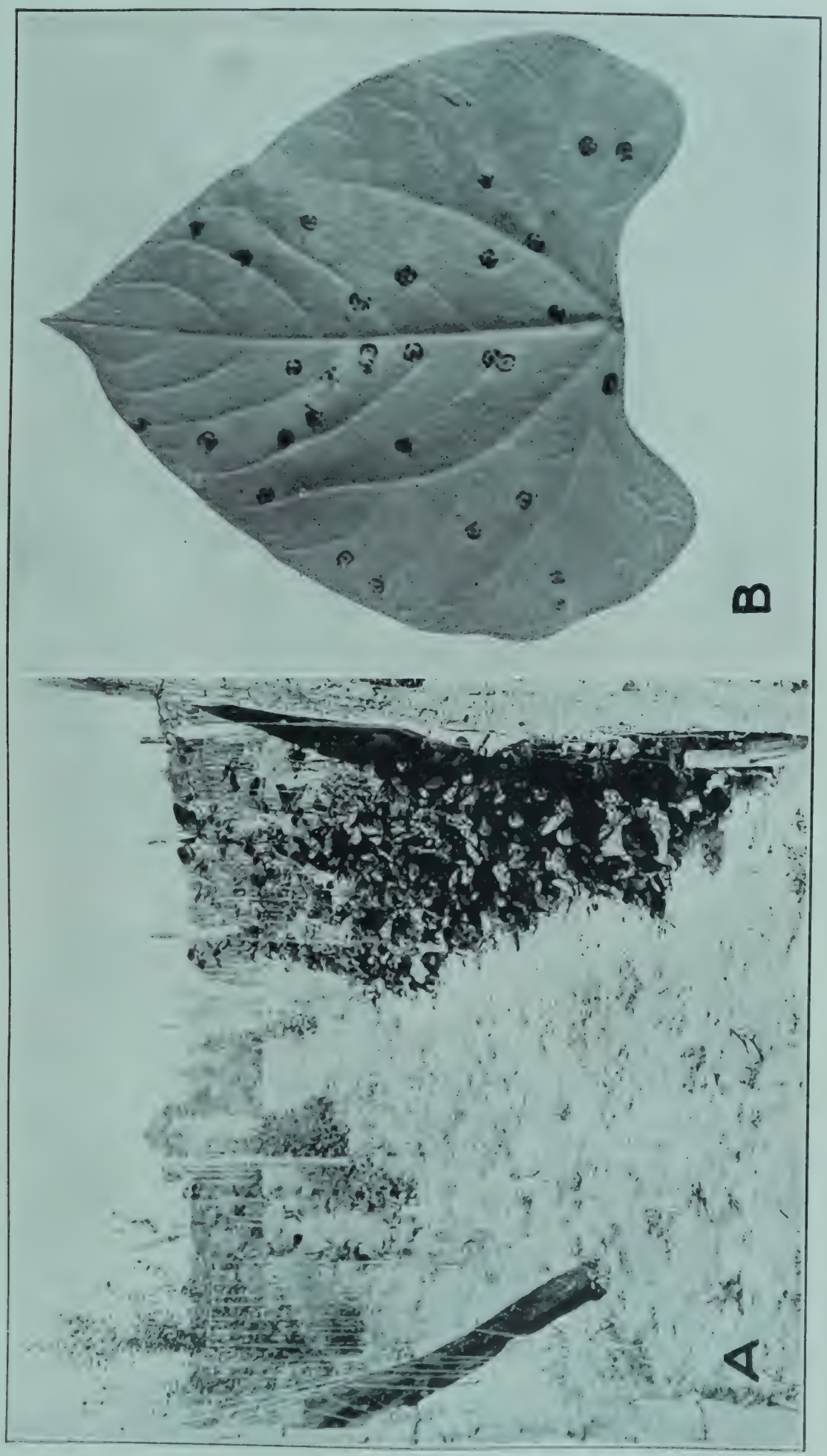
A.—Normal rust infection on a leaf of Dutch Case Knife developed in the greenhouse. Number of uredinia 544, leaf area 7.93 square inches, sori per square inch 68.6. Photographed 16 days after inoculation. Approximately natural size.

B. Abortive rust infection on a leaf of Improved Goddard. Number of flecks 140, leaf area 10.78 square inches, flecks per square inch 12.9. Photographed 16 days after inoculation. Approximately natural size.

PLATE 71

A.—Comparison of varietal susceptibility of beans to rust in the field. Left Powell's Prolific, susceptible, completely defoliated from rust infection. Right Tennessee Wonder, resistant, uninjured by rust. Photographed August 13, 1918.

B.—Secondary uredinia formed in circles around the primary uredinia on a leaf of Tennessee Green Pod. Photographed one month after inoculation in the greenhouse. Slightly reduced.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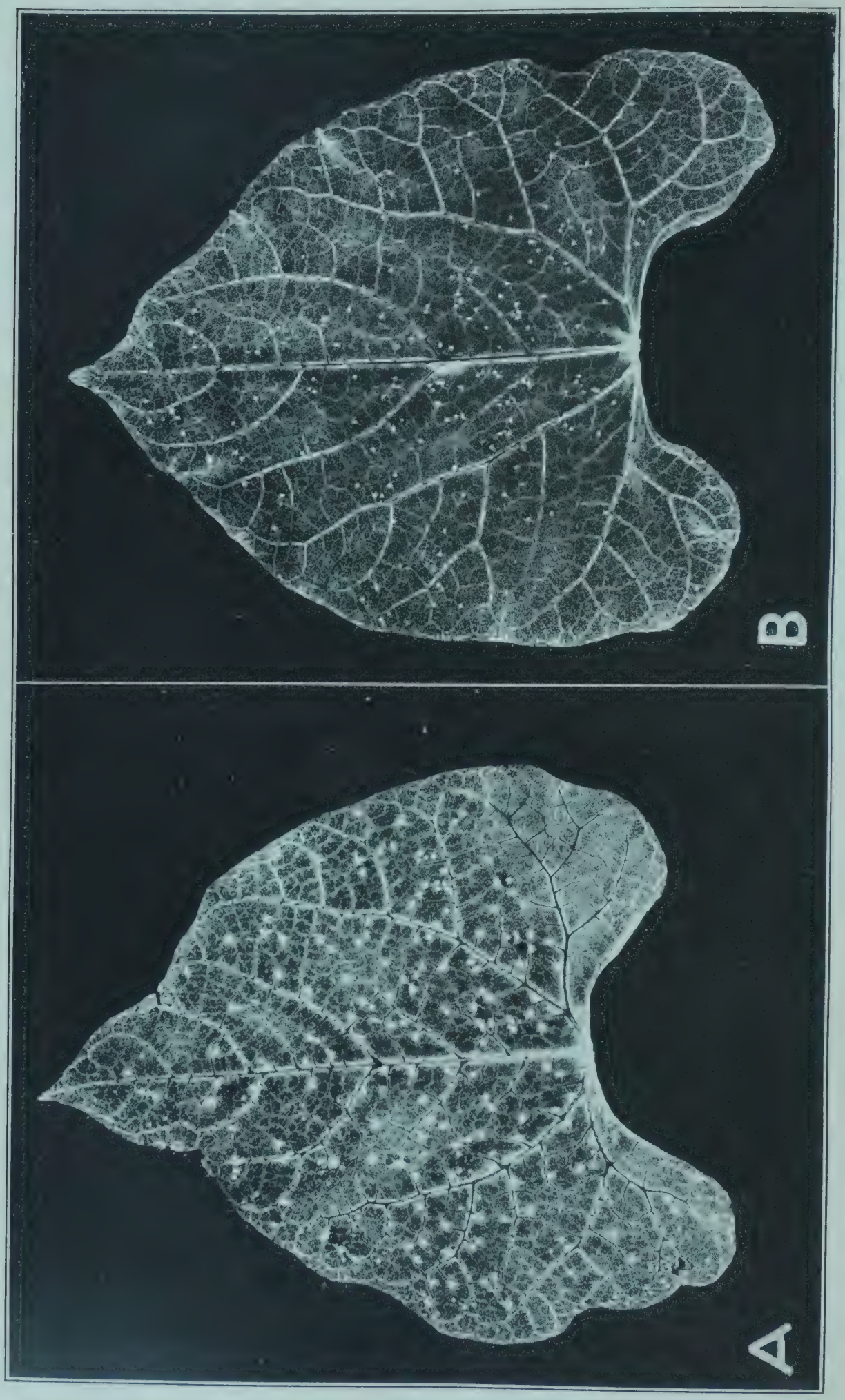
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PLATE 72

Contact prints of bean leaves showing comparative sizes of uredinia:

A.—Tennessee Green Pod. Average diameter of uredinia 600 μ, spore-producing capacity 100 per cent. Natural size.

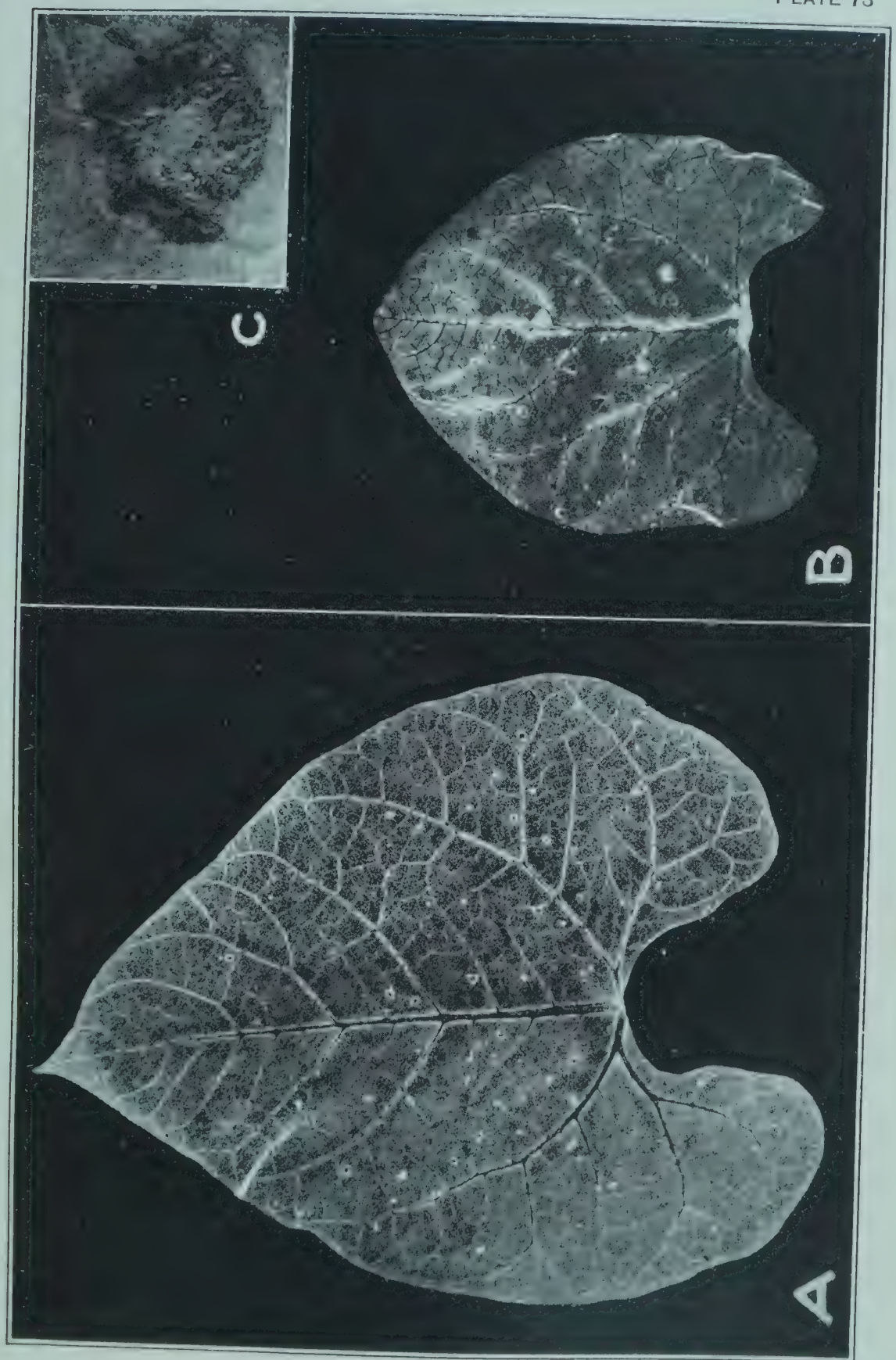
B.—Burpee's Stringless. Average diameter of uredinia 240 μ, spore-producing capacity 16 per cent. Natural size.

PLATE 73

A.—Contact print of a leaf of Mighty Nice showing small, angular flecks. Natural size.

B.—Contact print of a leaf of Crystal White showing unusually large, rounded flecks. Natural size.

C.—A typical fleck on a leaf of Tennessee Wonder. Approximately × 20.


C
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LIFE-HISTORY STUDIES OF THREE JOINTWORM PARASITES

By W. J. PHILLIPS and F. W. POOS, *Entomological Assistants, Cereal and Forage Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION[1]

The importance of the control of the jointworm (*Harmolita tritici* Fitch) by its parasites can not easily be overestimated. Up to the present time parasites have been the only means of control, and when these natural agencies fail whole fields of wheat often are destroyed. It is the opinion of the writers that effective control measures would have been put into practice or wheat growing in the Eastern States abandoned years ago had not the parasites of the jointworm in a large measure taken care of the situation.

The present system of crop rotation in the wheat-growing districts of the Eastern States furnishes ideal breeding grounds for the jointworm. This, however, is also true for the parasites; but we have learned by experience that we can not rely entirely upon the parasitic enemies of the jointworm to effect its control. It has been the common belief among scientists that the parasites check severe outbreaks of this pest within a few years, but the writers after studying closely a severe outbreak of the jointworm in Fauquier County, Va., have come to the conclusion that this is not always true. In that locality infestation by the jointworm has been very intense for several years, and according to observations of the past few seasons the numerical ratio of parasites to the jointworm has increased very slowly, until at the close of the year 1919 the jointworm is 92.6 per cent parasitized.

This paper treats of the life histories of three of the more important parasites of the jointworm. They are all hymenopterous parasites of the superfamily Chalcidoidea and have been mentioned frequently in literature, though no detailed accounts of their life histories as parasites of the jointworm have ever been recorded.

DISTRIBUTION

Ditropinotus aureoviridis Crawford and *Homoporus chalcidiphagus* Walsh and Riley are primary parasites, while *Eupelmus allynii* French was found to be both primary and secondary. The distribution of *D. aureoviridis* and *H. chalcidiphagus* is similar to that of the jointworm (fig. 1). *E. allynii* is commonly found wherever the Hessian fly

[1] The observations on which this paper is based were made at the United States Entomological Laboratory at Charlottesville, Va., during 1917, 1918, and 1919.

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(*Phytophaga destructor* Say) and the majority of the species of Harmolita are found. This includes practically all the wheat-growing regions of the United States. The writers feel that scientists in the past have given undue prominence to *E. allynii* as a primary parasite of the jointworm. *D. aureoviridis*, where it is normally found, occurs in greater abundance than either of the other two parasites treated in this paper.

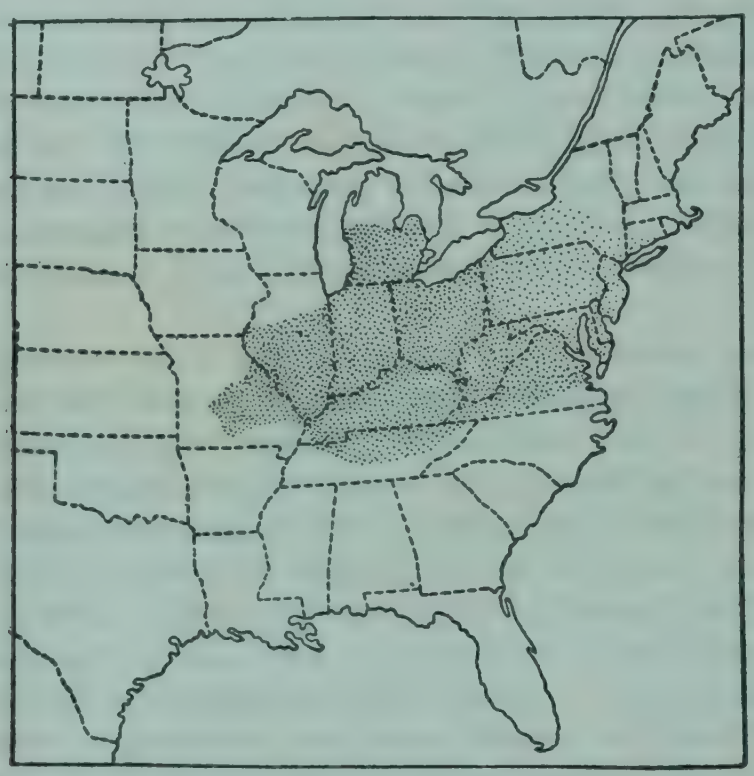


FIG. 1.—Present known distribution of the wheat jointworm in the United States.

METHODS OF REARING

The adult parasites used in this breeding work were obtained from material collected in various localities and kept through the winter at the laboratory at Charlottesville, Va., under as nearly normal conditions as could be provided. The parasites were placed in a cage (Pl. 79, A) which contained stock material of *Harmolita tritici*. This cage was a glass cylinder 9 by 14 inches, having a cheesecloth cover. It was placed upon a saucer of moist sand into which the stems containing galls of *H. tritici* were placed. The ordinary greenhouse wooden label was used for identification of the cages. Another very successful breeding cage (Pl. 74, A) which was used consisted of a lantern globe with a cheesecloth cover, placed upon a 5-inch pot. The parasites were given fresh straws for oviposition each morning and a few drops of dilute sugar water for them to feed upon. Feeding once a day seemed sufficient to supply the needs of the parasites. Oviposition was observed in these cages, and the stems were removed promptly and dissected. Eggs with their host were removed to glass cell cages [1] which were made for this

[1] PACKARD, C. M. LIFE HISTORIES AND METHODS OF REARING HESSIAN FLY PARASITES. *In* Jour. Agr. Research, v. 6, no. 10, p. 367–382, pl. 51–52. 1916.

purpose (Pl. 74, B). This cage was made by cutting window glass to the size of ordinary glass slides, and by the use of a dentist's small burr or carborundum grinder making a single small cell large enough to accommodate the larva of *H. tritici* in one surface of each slide. After a single egg with its host had been placed in each cell, the cell was closed with the ordinary glass cover slip, to which was applied a droplet of honey to hold it in place. The usual label was pasted on each slide for identification, and the slides were then placed in small closed pasteboard boxes in order to exclude the light. The life stages of the parasites from egg to adult could thus be observed under the microscope. Often, however, the parasite larva would crawl away from its host and starve during the night. Sometimes the host larva would turn dark as if decaying and another would have to be substituted. After long experience and with all possible care that could be given, it was found impossible to rear more than about 60 per cent of a given number of parasites by the cell-slide method. But since this method was the only one known in which the different stages could be observed continuously, it was adhered to throughout. *Eupelmus allynii* bred very freely by this method, while *Homoporus chalcidiphagus* bred less freely and *Ditropinotus aureoviridis* with some difficulty. The larvæ of the last-named species completed their development very readily, but very few continued through to the adult stage.

In order to have a control on the period of development of the parasites in the cell slides (Pl. 79, B), some of the eggs of the same age were not removed from the Harmolita cells but were permitted to remain and develop normally as they would in the field. It was found that each of these species very closely approximated the same period of development when reared in the glass cell as when reared in the galls of the jointworm under the same weather conditions.

In observing the larval development of these parasites it was comparatively easy to identify the various instars of *Ditropinotus aureoviridis* and *Eupelmus allynii* by the length, number, and position of the setæ and the change in size and shape of the larvæ. The instars were verified by making a balsam mount of each cast skin. It was found impossible to identify all of the instars of *Homoporus chalcidiphagus* except by removing to a balsam mount the entire contents of a glass cell, where the individual larva had developed, and noting the number of pairs of mandibles remaining therein.

All observations indicate that only one specimen of any of the three species studied ever matures in a single Harmolita cell. An individual host larva seems to furnish just enough food for a single parasite. Usually only the old dried skin of the host remains after the parasite larva has become full grown.

DITROPINOTUS AUREOVIRIDIS[1]

This species was first described, both male (fig. 2) and female (fig. 3), by Mr. J. C. Crawford in 1907.[2] In 1912, Mr. A. B. Gahan[3] described

FIG. 2.—*Ditropinotus aureoviridis:* Adult male. Greatly enlarged.

FIG. 3.—*Ditropinotus aureoviridis:* Adult female. Greatly enlarged.

the species *Ditropinotus flavicoxus*, basing his species on the very light coloration of the coxæ. Since publishing this description Mr. Gahan thinks it possible that *D. flavicoxus* may be only a varietal form of *D. aureoviridis*. The present writers have never reared a typical specimen

[1] Family Torymidae, Subfamily Monodontomerinae.

[2] CRAWFORD, J. C. NEW NORTH AMERICAN HYMENOPTERA. *In* Jour. N. Y. Ent. Soc., v. 15, no. 4, p. 178–179. 1907.

[3] GAHAN, A. B. DESCRIPTIONS OF TWO NEW GENERA AND SIX NEW SPECIES OF PARASITIC HYMENOPTERA. *In* Proc. Ent. Soc. Wash., v. 14, no. 1, p. 5–6. 1912.

of *D. flavicoxus*, though some specimens reared have much lighter-colored coxæ than those of the average *D. aureoviridis*.

HOSTS

This species apparently prefers *Harmolita tritici* and *H. vaginicola* Doane, and the writers have reared it in cell slides on *H. elymicola* Phillips and Emery and *H. grandis* Riley. It probably will parasitize any of the gall-forming species of Harmolita, though the writers have reared it only from field collections of *H. tritici*, *H. vaginicola*, *H. grandis*, *H. atlantica* Phillips and Emery, and *H. secalis* Fitch. It breeds freely upon the larvæ of *Eurytoma* spp. in the field and in breeding cages, and upon one occasion an egg was deposited in a Harmolita cell upon a pupa of *Ditropinotus aureoviridis*. It undoubtedly breeds upon *Homoporus chalcidiphagus* also, though it plays the rôle of secondary parasite only when the individuals of Harmolita are very highly parasitized.

EGG

The eggs (Pl. 75, A; 77, B) are deposited in the gall-like cells of *Harmolita tritici* external to the host larva. They are not always placed directly upon the host, but the small size of the cell of the jointworm requires that the egg be in close proximity to its host. The egg is grayish white in color and opaque. It is elongate, kidney-shaped, but asymmetrical. There is a nipple-like process at each end of the egg. The surface of the egg is covered uniformly with spicules with the exception of the nipple-like process at the posterior end. The average of four eggs measured 0.6772 mm. in length and 0.1583 mm. in greatest width.

The period of incubation of 199 eggs in cell slides varied at different times during the breeding season from 1 to 5.5 days. The average time was 3 days. Low temperatures greatly retarded development. At the time the egg is ready to hatch, the mandibles of the young larva can be observed at the large end of the egg, where they move back and forth occasionally. Sometimes the whole outline of the head can be seen through the chorion just before the young larva cuts its way out with its mandibles. The round nipple-like process extended out beyond the larva's head makes it look as though it were wearing a dunce cap. The larva keeps pushing forward with its head against the flexible shell, at the same time slashing away with its mandibles until finally the shell is ruptured. The larva then forces its head through the aperture (Pl. 75, B) and begins feeding at once upon the host larva. Several hours are usually required for the larva to extricate itself from the eggshell.

LARVA

First instar (Pl. 75, C).—The color of the first-instar larva is translucent whitish. Just before the first molt the average length of three larvæ was 0.9152 mm. and the average greatest width was 0.2060 mm.

When hatched the larva is very slender and gradually tapers toward the posterior extremity. At this time the head (Pl. 75, D) is considerably larger than the individual body segments. Just before the first molt is cast (Pl. 75, E) the head is much narrower than any of the first 8 body segments. The head is convex anteriorly and bears 7 pairs of rather long setæ and a pair of fleshy cylindrical tubercles which apparently are antennæ. The mandibles of this stage (Pl. 76, A) appear to be very highly chitinized, very slender and pointed. The body consists of the usual 13 segments bearing 5 pairs of spiracles, a pair on each of the last 2 thoracic and first 3 abdominal segments. There are 2 lateral rows and 2 subdorsal rows of long setæ, 1 seta to each row to each body segment, and 2 subventral rows of long setæ on the thoracic segments only; in addition, around each segment there are 2 or more irregular rows of minute setæ.

SECOND INSTAR (Pl. 75, F).—Color as in previous instar. Average length of three larvæ 1.1645 mm. and average greatest width 0.3473 mm. In this instar the larva is pointed at each extremity although very much more pointed posteriorly. The head is convex anteriorly and bears 5 pairs of setæ; antennæ as in previous instar. The mandibles (Pl. 76, B) have changed shape entirely; they are very broad at the base and much less heavily chitinized than in the previous instar. There are 9 pairs of spiracles present, 1 pair to each of the last 2 thoracic and first 7 abdominal segments. The short, minute setæ present in the first instar are not present in this or any of the succeeding instars, and the long setæ are shorter but are arranged on the body as in the previous instar.

THIRD INSTAR (Pl. 75, G).—Color same as in previous instar; average length of three larvæ 1.3533 mm. and average greatest width 0.4083 mm. Shape is much the same as in the previous instar. Head bears 8 pairs of setæ, which are longer than in the previous instar; antennæ same; mandibles (Pl. 76, C) much longer and more slender and about as heavily chitinized as in the previous instar. The setæ of the body are much more numerous and are longer than in the previous instar. The first segment bears a partial double row of setæ around it; the other segments bear an irregular single row of setæ of unequal length around them.

FOURTH INSTAR (Pl. 75, H).—Color and shape as in previous instar; average length of three larvæ 2.1233 mm. and average greatest width 0.5833 mm. Head as in previous instar except that setæ have become much longer and more numerous; antennæ same; mandibles (Pl. 76, D) same general shape as in previous instar, but larger. The setæ of the body are more numerous and are longer than in the previous instar, There are three incomplete, irregular rows of setæ around the first thoracic segment; two rather complete, irregular rows around the second; an irregular single row around the last thoracic and all the abdominal seg-

ments with the exception of the ninth, which bears a partial double row irregularly placed. Setæ are of uneven length as in previous instar

FULL-GROWN LARVA (fig. 4).—Color: Larva has a slight ferruginous tinge, due to the setæ; the body is dirty whitish with a dark central area, due to the contents of the digestive tract; the average length of 5 larvæ was 3.108 mm., and the greatest width averaged 1.106 mm. In this stage the larva is less pointed, broader, and thicker and much more hairy than at any previous stage; setæ cover the entire surface of the head except for a narrow line down the center of the face; there also seems to be a slightly chitinized area down the face on each side of this median line. Antennæ apparently the same as in previous instars. Mouthparts (Pl. 76, F): Mandibles (Pl. 76, E) same as in previous instar with the exception that they are considerably larger. Labrum consists of a simple triangular piece and is only very slightly chitinized; maxillæ and labium exist only as fleshy lobes slightly chitinized on their surface

FIG. 4.—*Ditropinotus aureoviridis:* Full-grown larva. × 24.5.

and bear 8 setæ and 4 groups of slight elevations or tubercles. (See Pl. 76, F.)

The setæ of the body are longer and much more numerous than in the previous instar; each segment except the last four abdominal bears an irregular row of very long setæ around it; in addition, the first thoracic segment bears a second irregular row of varying length around it; the second thoracic segment bears a second row likewise and a partial third around it; the third thoracic segment bears only one additional row around it; the first abdominal segment bears a partial second row; the second, third, fourth, fifth, and sixth the same. The seventh, eighth, and ninth bear numerous setæ, varying greatly in length, that can scarcely be designated as constituting definite rows; the last segment bears numerous small setæ and is invaginated laterally.

In order that the number of larval instars might be ascertained, the larvæ were kept under almost constant observation under the microscope night and day from the time they were hatched until they became full-grown. By this method there appeared to be four fairly distinct larval molts, as indicated by the external changes of the larvæ. These observations were verified by making balsam mounts of the cast skins of

the individual larvæ and noting the number of pairs of mandibles. Five pairs of mandibles, gradually increasing in size (Pl. 76, A–E), were thus found, seeming to give definite proof of the accuracy of the observations.

As soon as the young larva hatches, it pushes its head out of the egg-shell (Pl. 75, B) and immediately begins to feed. It often feeds thus for about 24 hours before it completely extricates itself from the shell. In this stage it often feeds with only its head touching the host. It apparently slashes away with its mandibles until it has made a slight incision in the body wall of the host, whereupon it begins to suck up the body fluids. The larva feeds almost continuously, if undisturbed, until nothing remains of the host larva but the empty skin. When the parasites are very abundant, very often several eggs are found in a single jointworm cell, but never more than one larva completes its development therein. The largest and strongest larva evidently overcomes and destroys the others. In moving forward over its host the tip of the abdomen of the parasitic larva is touched to the surface of the host, where it adheres. It seems to function as an anal proleg and to enable the larva to bow the body upward. Simultaneously each segment moves forward as far as possible, and the sucker-like mouth is touched to the surface of the host, where it adheres and acts as an anchor by means of which the larva pulls the body forward. By alternating these movements the larva is enabled to move over its host with comparative ease.

Just before each molt the body of the larva is considerably larger than the head. This fact enables one to determine with a fair degree of accuracy when the larva will molt. This character is much more apparent in the early stages. The later stages are indicated more clearly by the increased number of setæ. The duration of instars, as is common with most insects, varies with the temperature, hot weather accelerating and cooler weather retarding development. The first instar lasts from one to four days; later instars approximate this period of time, varying directly with temperature and the condition of the host.

Larvæ of this species which were reared in glass cells became full-grown in from 6 to 24 days. One hundred and thirty-seven larvæ observed for this purpose at various times during the breeding season became full-grown within an average of 11 days.

During the season of 1917, 41 larvæ became full-grown in glass cells, but died during the winter before any of them pupated. In 1918, out of more than 100 larvæ, which became full-grown in glass cells, only 9 pupated during the same season. Those that pupated did so within a period of from 4 to 17 days after they had completed feeding. These individuals were of the first and second generations. Sixteen adults emerged from the remaining material, and several others pupated. Both first- and second-generation larvæ completed development the following spring. Five of this group were kept in the laboratory, and the remaining larvæ were kept in a cellar during the winter. Cage material

reared in Harmolita galls as a control on those reared in glass cells for the purpose of determining how well the glass cells approximated normal conditions for these parasites, for that particular season, showed that many larvæ of the first generation did not pupate during that season. This was found to be true likewise of *Homoporus chalcidiphagus*.

PREPUPA

The prepupal stage (Pl. 77, A; fig. 5) in three specimens observed varied from 1 to 6 days, with an average of 2 days. The larva does not void excrement during its earlier stages of development, but when the full-grown larva is ready to pupate, a considerable amount of excrement is voided and the specimen contracts considerably. The first four segments of the abdomen contract equally until each is somewhat less than half its usual length in the full-grown larva. If the specimen is viewed from the ventral aspect, the last two segments of the abdomen appear

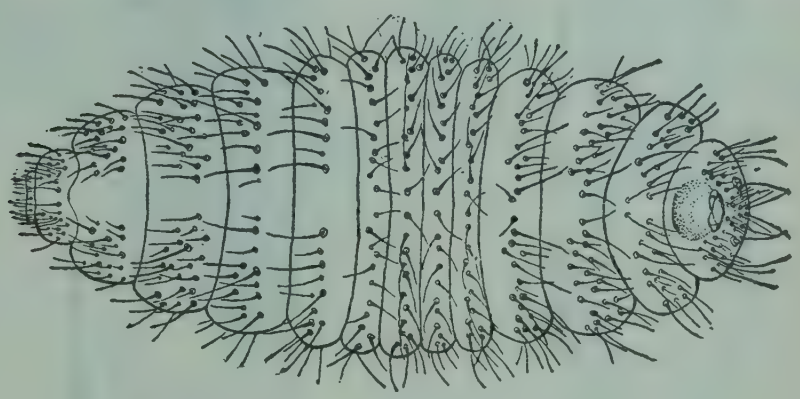


FIG. 5.—*Ditropinotus aureoviridis:* Prepupal stage. × 34.

to curve backward dorsally. The measurements of two prepupæ averaged 2.2050 mm. in length and 0.9450 mm. in greatest width.

PUPA

Pupation was observed upon one occasion, the process requiring 17 minutes. The fully developed pupa was plainly visible through the old larval skin, which first split down the center of the head, and the pupa then gradually worked itself forward through the opening. At first the pupa is a very delicate brown in color, gradually changing and becoming very dark, approximating the color of the adult insect. The pupal stage varies in length from 8 to 15 days. The average for 8 specimens observed in glass cells was 10 days. Six female pupæ (Pl. 77, C; fig. 6) averaged 3.0479 mm. in length and 0.8108 mm. in greatest width. One male pupa (Pl. 77, D; fig. 7) measured 2.66 mm. in length and 0.91 mm. in greatest width.

ADULT

No males normally occur in the first generation of this species, and the females seem greatly to outnumber the males in succeeding generations. They pass the winter as full-grown larvæ in the gall-like cells

of the jointworm, and the first generation of this parasite begins to emerge about the first week in June and the second generation about the first week in July in Virginia, according to the authors' records. Previous to the season of 1918 it was supposed that there were only two generations each year; but during 1918 and 1919 two complete and a partial third generation were reared in glass cell slides,[1] and apparently a partial third one was reared from the field in 1919. There appeared to be only two main generations annually in the field, and further observations, therefore, are necessary before it can be determined definitely whether there is normally a partial third generation under field conditions.

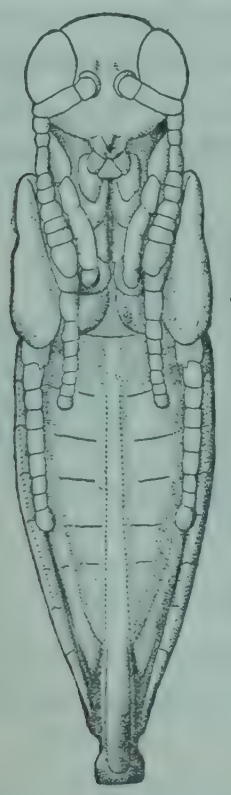


FIG. 6.—*Ditropinotus aureoviridis:* Pupa of female. × 25.

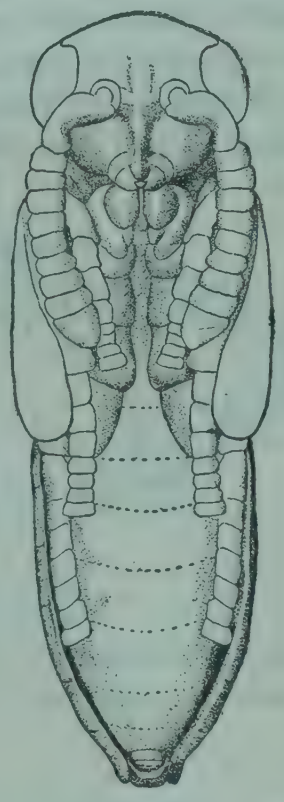


FIG. 7.—*Ditropinotus aureoviridis:* Pupa of male. × 28.

The largest number of eggs secured from a single female was 18 during a period of 12 days. One female lived 48 days. When the female is ready to oviposit she walks up and down the wheat stem, tapping it with her antennæ until a jointworm gall is located. After locating a suitable cell the female brings the abdomen almost at right angles to the thorax, at the same time elevating the body as much as possible. She then touches the tip of the ovipositor to the surface of the wheat stem. In this position she uses her ovipositor very much as a gimlet until it is forced into the Harmolita cell, at which time the body assumes its more nearly normal position. The egg, after oviposition, has the same shape as before oviposition. Five to 10 minutes are required for the female

[1] This observation was repeated in 1920 with the same results.

to deposit one egg. The females have been observed ovipositing at night in the breeding cages, but the greatest activity occurs during daylight.

HOMOPORUS CHALCIDIPHAGUS[1]

This species was first described, male and female (fig. 8), in 1869.[2] This is one of the oldest recorded parasites of the jointworm and has been reported many times in literature, though the detailed account of its life history has not as yet appeared in print.

Next to *Ditropinotus aureoviridis* it is the most important parasite of *Harmolita tritici* in Michigan, Illinois, Indiana, Ohio, Kentucky, Tennessee, and Missouri. In the Atlantic States *Eurytoma* sp. is probably of greater importance than *H. chalcidiphagus;* in fact, at some points *Eurytoma* spp. undoubtedly ranks first in importance of the parasites of *H. tritici. H. chalcidiphagus* undoubtedly suffers more from the depredations of secondary parasites than any of the other primary parasites of the jointworm that have come under the observation of the writers. As evidence of this fact, *H. chalcidiphagus* was very abundant at Warrenton, Va., when the status of the various parasites was first studied there, whereas at the present time it scarcely exists in this locality. This is true in spite of the fact that there are five generations a year of

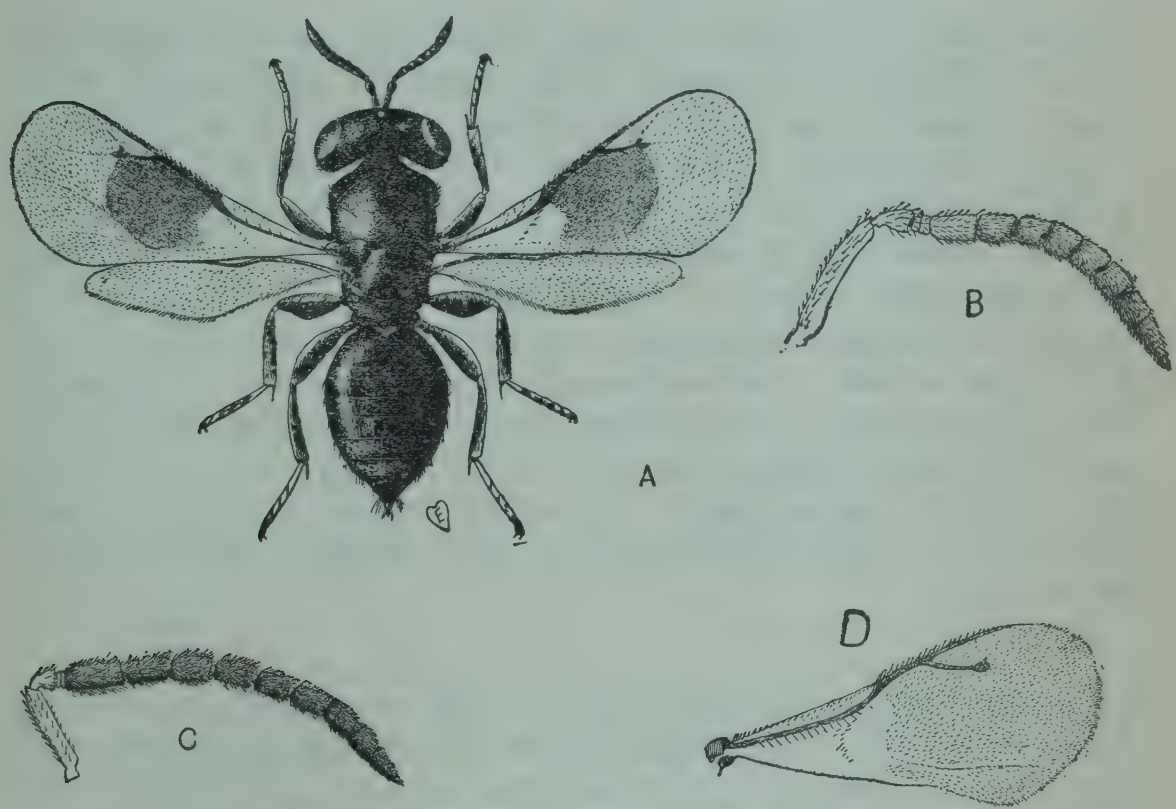


FIG. 8.—*Homoporus chalcidiphagus:* A, Adult female; B, antenna of adult female; C, antenna of adult male; D, wing of adult male.

[1] Family Pteromalidae, Subfamily Merisinae.

[2] WALSH, Benjamin D., and RILEY, Charles V. THE JOINT-WORM (ISOSOMA HORDEI HARRIS). *In* Amer. Entomologist, v. 1, no. 8, p. 152. 1869.

this parasite in the vicinity of Charlottesville. *Eupelmus allynii* is undoubtedly largely responsible for this condition of affairs, although *D. aureoviridis* very probably assists greatly in bringing about the condition which has been noted above. The larvæ of *H. chalcidiphagus* are very smooth and inactive and apparently are as easy prey to other parasites as are the Harmolita larvæ. *Eupelmus allynii* and *D. aureoviridis*, on the contrary, are very active and therefore can defend themselves to a large extent. This is particularly true of *D. aureoviridis*, which is very hairy and very active and usually can crush the egg of other parasites before hatching is possible.

HOSTS

Homoporus chalcidiphagus has been reared from field collections of the following species of Harmolita: *tritici*, *vaginicola*, *secalis*, *hordei* Harris, *elymicola*, and *atlantica*. It is a primary parasite of the jointworm. During 1918 a number of experiments were conducted in order to learn whether it could be induced to breed as a secondary parasite. In most of these experiments it refused absolutely to oviposit upon any larvæ except Harmolita, though in a few instances it oviposited upon *Eurytoma* sp. and in a single instance one larva developed upon a pupa of *Ditropinotus aureoviridis*. In some instances when the larva of *Eurytoma* sp. and the egg of *H. chalcidiphagus* were transferred to cell slides the egg was crushed by the activity of the larva. From this it would appear that when *H. chalcidiphagus* happens to oviposit in Harmolita cells containing larvæ of *Eurytoma* sp. in the field, a large part of these never reach maturity.

EGG

The egg (fig. 9) is elongate, slender, kidney-shaped, slightly larger at the anterior end. It is circular in cross section and without pedicel or flagellum. It is white in color, and the surface is very smooth. The average length of 4 eggs was 0.4204 mm. and the greatest width 0.1347 mm. They are always found external to the host. Sometimes as many as 4 or 5 were found in one cell of *Harmolita tritici*, but never more than one larva completed its development when more than one egg was placed upon a single host. Some of the eggs for some cause or other shriveled up and failed to hatch. This was not due to handling, since the eggs were found to be shriveled when the jointworm cells were opened. In hatching, the larva ruptures the large end of the egg and begins feeding before it completely emerges from the shell. The period of incubation in glass cells varied from 1 to 4½ days, depending upon weather conditions, warm weather accelerating and cool weather retarding development. The average length of the egg stage of 71 eggs observed was about 2.5 days.

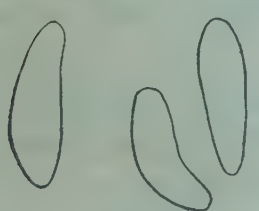


FIG. 9.—*Homoporus chalcidiphagus*: Eggs. × 47.5.

LARVA

First instar (fig. 10).—Newly hatched larvæ are translucent whitish in color; the average length of three larvæ in this stage was 0.3945 mm. and the greatest width, 0.1490 mm. When hatched the larva tapers toward each extremity but is pointed posteriorly; it is very smooth, the setæ being so small that it is practically impossible to determine the exact number. There is a pair of prominent fleshy elevations or tubercles on the front of the head that are apparently the antennæ. The head is very smooth, and if any setæ are present they are so small that it is almost impossible to ascertain their number and position. The mandibles (Pl. 76, G) of the first instar are simple hooks, very slightly chitinized, very pointed at tips, and broad at the base. The body consists of the usual 13 segments and bears 5 pairs of spiracles, a pair to each of the last 2 thoracic and first 3 abdominal segments. The external changes in the succeeding instars were so slight that it was impossible to identify them accurately. A balsam mount was made of the entire contents of one of the glass cells where an individual larva had developed and 5 pairs of mandibles (Pl. 76, G–K) were found. As all of these pairs gradually increased in size it is reasonable to suppose that they were those of *Homoporus chalcidiphagus*. One pair (the fourth) differs slightly in shape from the other four pairs, though in size it fits in as the fourth pair. The slightly different shape of the fourth pair of mandibles is probably due to the fact that the mandibles are difficult to mount so as to have all the pairs in the same relative position, and probably the fourth pair was viewed from a slightly different angle when it was illustrated. No illustrations other than those of the mandibles were made of any of the larval instars, except the first and the full-grown larvæ, since none of the other instars could be determined accurately.

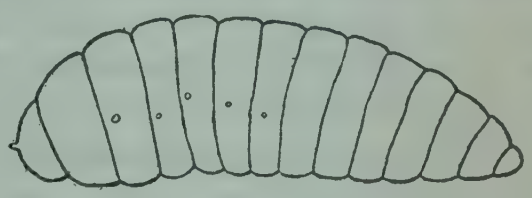


Fig. 10.—*Homoporus chalcidiphagus:* Lateral view of first-instar larva. × 128.

Full-grown larva (Pl. 74, D; fig. 11).—Color dirty whitish, with a dark line showing through the center of the larva due to the contents of the digestive tract. The average length of five full-grown larvæ was 3.08 mm., and the greatest width was 0.91 mm. The full-grown larva is of the same general shape as the first-instar larva. The last abdominal segment is clearly invaginated laterally. The head (Pl. 76, M) is very smooth and bears a pair of rather prominent fleshy elevations or tubercles on the front that are evidently the antennæ. It also bears four pairs of very small setæ—one pair laterad of the mandibles; a pair laterad of these, about the middle of the cheek; a pair dorsad of these; and a fourth pair above and inside the antennal region. There are three pairs of minute

setæ just above the labrum. Mouthparts (Pl. 76, L) and mandibles (Pl. 76, K) are the same general shape as in the first instar, though much larger and somewhat more heavily chitinized. The labrum is a small fleshy triangular piece. The labium and maxillæ are fused and exist only as fleshy lobes bearing three pairs of minute setæ and four pairs of very slight elevations or tubercles.

The body bears 9 pairs of spiracles, a pair to each of the last 2 thoracic and first 7 abdominal segments; the setæ are so small that it is almost impossible to locate them definitely, although there are apparently 4 rows on the ventral surface of the 3 thoracic segments, 2 lateral rows, and 2 subdorsal rows extending the entire length of the body, 1 seta on each segment in each row, with the exception of the last abdominal segment which bears 2 pairs of subdorsal setæ. As indicated previously, the 5 pairs of mandibles found in the cell slide where an individual of

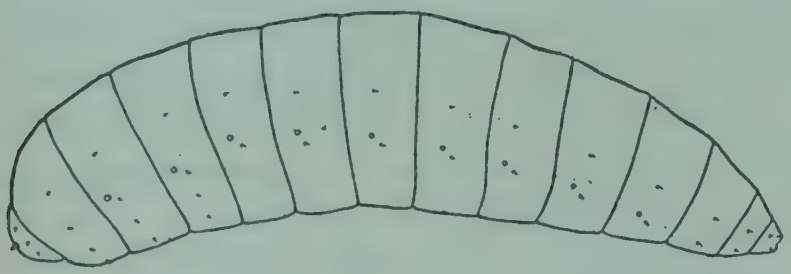


FIG. 11.—*Homoporus chalcidiphagus:* Lateral view of full-grown larva. × 24.7.

this species had pupated indicate that there are four larval molts during the development of this species.

The feeding habits of this larva are very much the same as those of *Ditropinotus aureoviridis*, though the larvæ are very much more delicate and less active; consequently, they are more difficult to rear to full-grown larvæ. Observations on 42 larvæ of this species showed that they became full-grown in glass cells in from 5 to 25 days, depending upon weather conditions and the condition of the host. During the summer they developed very rapidly, while in the fall the period was greatly lengthened. The average period of development was about 11 days. The average period of time between the full-grown larva and the prepupal stage for 40 individuals was 4½ days. In a large number of cases the larvæ became full-grown and remained quiescent for many months. Many then contracted slowly and died, while others completed their development to adults the following season. In one instance 2 larvæ remained in the quiescent stage from August, 1917, until September, 1918, when they finally died. At the present time, December, 1919, the writers have living larvæ in the quiescent stage which became full-grown larvæ as early as June, 1918. The winter is passed as full-grown larvæ in the cells of the jointworm.

PREPUPA

The prepupal stage (Pl. 74, C; fig. 12) of the larva is indicated by the contraction of the anterior abdominal region, the greatest constriction occurring in the first 2 abdominal segments which contract to about one-half the original width. In this stage a large amount of excrement is voided and the prepupa is then pure white in color. No excrement is voided during any period of larval growth. Observations on 37 individuals showed that they remained in this stage from 1 to 6 days, the average being about 2 days. The average length of three prepupæ was 2.4966 mm. and the greatest width averaged 0.8633 mm.

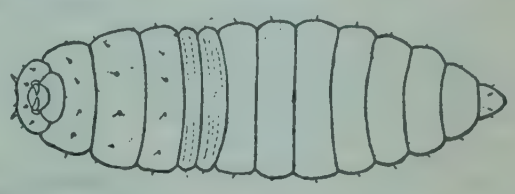


FIG. 12.—*Homoporus chalcidiphagus*: Ventral view of prepupa. × 20.

PUPA

The pupa (Pl. 74, E–G) of this species is at first pure white. The eyes first begin to turn pink and gradually grow darker as the remaining portions of the body become dark. The entire pupa finally becomes bluish black, approximating the color of the adult. The length of the pupa stage was found to vary from 5 to 23 days for individuals pupating during the same breeding season. The average length of the pupal period for 64 individuals was between 9 and 10 days. The average length of 3 female pupæ was 3.5 mm. and the greatest width averaged 0.7933 mm.; the average length for 3 male pupæ was 2.7533 mm.; and the greatest width averaged 0.7933 mm. The male pupa can easily be distinguished from the female pupa by the fact that the antennæ extend the full length of the tarsi of the forelegs. This is not true of the female pupa.

ADULT

The adult (fig. 8) emerges in Virginia during the latter part of May and continues breeding up into the month of October. Even in the northern States on warm days in October this parasite has been observed in the fields. Five complete generations were reared at this laboratory in cell slides from May to September in 1918. These observations accorded with rearings in Harmolita galls in wheat stems kept under similar conditions.

Males normally occur in about equal numbers with females, though this species will breed parthenogenetically, in which case the offspring are males.

The average number of eggs deposited for 5 individual females was 31.8, for an average period of 15.2 days. One individual deposited 45 eggs during a period of 17 days. One individual lived for 31 days. The

females always took up a position with the head directly up the stem in ovipositing. The process of oviposition was very much the same as described for *Ditropinotus aureoviridis*. The shape of the egg of *Homoporus chalcidiphagus* is the same after oviposition as before.

EUPELMUS ALLYNII[1]

This species (figs. 13, 14, 15) was first described in 1882.[2] In 1916 Mr. C. M. Packard[3] gave an excellent account of the life history as a

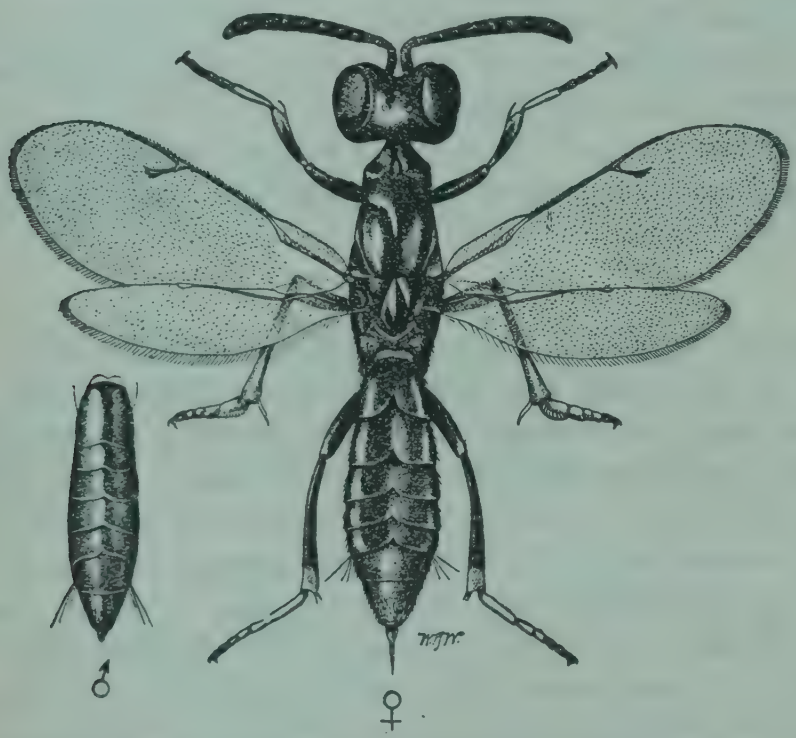


FIG. 13.—*Eupelmus allynii:* Adult female and abdomen of male, greatly enlarged. (Webster.)

parasite of the Hessian fly, and his paper is the only published record on the detailed life history of this species. It has been considered by some

FIG. 14.—*Eupelmus allynii:* Lateral view of adult female, greatly enlarged. (Webster.)

writers as the most important parasite of the jointworm. In the estimation of the present writers it ranks about fourth in importance in the list of parasites of *Harmolita tritici*.

[1] Family Encyrtidae, Subfamily Eupelminae.

[2] FRENCH, G. H. THE WHEAT-STRAW WORM. (ISOSOMA ALLYNII, FRENCH.) *In* 11th Rpt. State Entomologist Ill. [1881], p. 73–81. 1882.

[3] PACKARD, C. M. LIFE HISTORIES AND METHODS OF REARING HESSIAN-FLY PARASITES. *In* Jour. Agr. Research, v. 6, no. 10, p. 367–382, pl. 51–52. 1916.

HOSTS

Eupelmus allynii is both a primary and a secondary parasite of the jointworm. It apparently will breed as freely as a secondary parasite in jointworm cells as upon jointworms themselves under both field and cage conditions. It has been found breeding as a secondary parasite on *Ditropinotus aureoviridis*, *Homoporus chalcidiphagus*, and *Eurytoma* sp. both in cages and in the field. We have reared it in connection with other parasites from the following species of Harmolita from field collections: *tritici*, *vaginicola*, *maculata* Howard, *elymicola*, *elymivora* Phillips and Emery, *atlantica*, *albomaculata* Ashmead, *grandis* form *grandis*, and from what is thought to be a new species which forms galls in *Panicum clandestinum* L.; also probably from *H. occidentalis* Phillips and

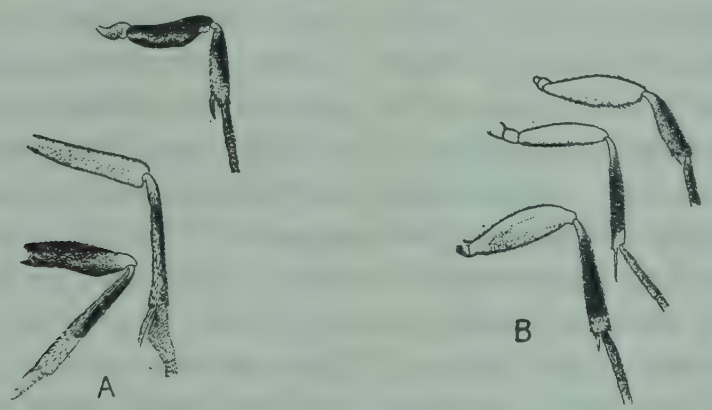


FIG. 15.—*Eupelmus allynii:* A, Legs of female; B, legs of male; greatly enlarged.

Emery, and *H. hesperus* Phillips and Emery. In the majority of these instances it can not be said positively whether or not it was breeding as a primary parasite. It may have been and probably was breeding as both a primary and a secondary parasite in each instance. It will probably breed upon any species of the genus Harmolita.

EGG

The egg (Pl. 77, E, F; 78, A) of this species is perfectly smooth without any sculpturing whatever and is white in color. The average length of four eggs was 0.4109 mm., and the greatest width 0.1578 mm. It is ellipsoidal in shape; the chorion is thin and elastic; there is a pedicel at the cephalic pole of the egg that is nearly as long as the egg itself and a slender flagellum at the opposite pole that is scarcely one-fourth as long as the pedicel. The pedicel is usually folded back against the egg after oviposition.

The egg is always deposited external to the host. About 50 per cent of the eggs found were fastened to the wall of the Harmolita cell by means of a delicate, fibrous, netlike structure which was apparently woven from fine white threads (Pl. 77, E). When this covering was found over the eggs it was usually fastened down rather firmly all around

the edges, holding the egg securely in place. As many as eight eggs have been found in one Harmolita cell, in which case there was always a separate covering for each egg, though these coverings were usually attached to each other.

The period of incubation of 79 eggs varied from 1 to 4.5 days during the breeding season, depending upon weather conditions. The average length of the incubation period for these 79 eggs was 2.4 days.

In the process of hatching it was observed that the head of the larva broke through the chorion near the base of the pedicel. The eggshell is apparently ruptured in the same manner as is that of *Ditropinotus aureoviridis*, previously described (p. 409).

LARVA

First instar (Pl. 78, B, C).—The newly hatched larva is translucent whitish in color; the average length of three larvæ was 0.4208 mm. and the greatest width 0.1315 mm. The larva tapers toward each extremity, the greatest diameter being about the third thoracic segment; the larva is pointed posteriorly. Just before the first molt is cast the head is much narrower than any of the first 8 body segments. On the front of the head are two rather long fleshy protuberances which very probably are the antennæ. The antennæ in this instar are fully as long as in any of the succeeding instars, if not longer. There are four pairs of rather prominent setæ on the head, one pair just above and inside the antennal area, two pairs above the mandibles, and a pair laterad of the antennæ. The mandibles (Pl. 76, N) in this stage are simple hooks which are very highly chitinized and very slender and pointed. The whole head shield is more highly chitinized than in later instars. The body is composed of the usual 13 segments which bear 5 pairs of spiracles, a pair to each of the last 2 thoracic and first 3 abdominal segments. There are two rows of subdorsal setæ the full length of the body that are extremely prominent, giving the larva almost the appearance of an Indian war bonnet when seen in profile (Pl. 78, B, C). There are 2 lateral rows in which the setæ on the first 2 thoracic segments are exceedingly prominent, being nearly three times the length of any of the remaining setæ in these rows. There are also 2 subventral rows on the thoracic segments only. In addition to the setæ just described, each segment is rather densely covered with very minute setæ which are not present in the succeeding instars.

Second instar (Pl. 78, D).—The color and general shape are the same as in the previous instar, except that the abdomen is not so pointed. The head of the larva is much blunter anteriorly than in the previous instar. Three larvæ in this instar averaged 1.050 mm. in length and 0.4316 mm. in greatest width. The antennæ are slightly less prominent and the setæ very much less prominent than in the previous instar.

There are 5 pairs of small setæ on the head—a pair just above the mandibles, a pair laterad of these, a pair dorso-mesad of the antennæ, a pair caudo-dorsad of the antennæ, and a pair on top of the head. The mandibles (Pl. 76, O) have changed shape considerably and are now much broader at the base and are less slender and less chitinized than in the previous instar. The labrum bears 4 heavily chitinized lobes or denticles. The body bears 9 pairs of spiracles, a pair being borne on each of the last 2 thoracic and first 7 abdominal segments. The setæ on the body in this instar are very small and inconspicuous. There are 2 subdorsal rows, 2 lateral or spiracular rows, and 4 subventral rows on the thoracic segments; also 4 additional subdorsal setæ on the first thoracic segment and 2 subventral setæ on both the eighth and ninth abdominal segments.

THIRD INSTAR (Pl. 78, E).—The color and general shape are very much as in the previous instar. Four larvæ in this instar averaged 1.610 mm. in length and 0.4925 mm. in greatest width. The setæ on the head are slightly more prominent. There are 5 pairs of setæ located as in previous instar. Antennæ as in previous instar. Mandibles (Pl. 76, P) with general shape same as in previous instar, although they have increased considerably in size. The labrum in this instar has 4 prominent chitinized denticles or lobes and a rather inconspicuous one at each side of these. Spiracles as in previous instar. Between the last 2 thoracic, the last thoracic and first abdominal, first and second, and second and third abdominal segments there are rather prominent, fleshy, dorsal folds. The setæ are slightly more prominent and are arranged as in previous instar, except for 1 additional subventral pair on first thoracic segment, and an additional pair of spiracular setæ on the sixth and seventh abdominal segments.

FOURTH INSTAR (Pl. 78, F).—Color, general shape, head, antennæ, and setæ of head as in previous instar, except that the setæ are much more conspicuous. Two larvæ in this instar averaged 1.855 mm. in length and 0.6300 mm. in greatest width. The mandibles (Pl. 76, Q) in this instar are stouter and more heavily chitinized than in the previous instar. There are 4 heavily chitinized lobes to the labrum with an inconspicuous lobe on each side of them. Spiracles as in previous instar. The dorsal fleshy folds same as in the previous instar with an additional one between the third and fourth abdominal segments. This character is probably somewhat dependent upon the position the larva is in at the time of observation. The body setæ are slightly more prominent than in the previous instar. There are 14 setæ on the first thoracic segment, 8 on both the second and third thoracic segments; 4 setæ on each of the first 5 abdominal segments, 6 on each of the sixth, seventh, eighth, and ninth abdominal segments, 4 on the tenth. The setæ are arranged as shown in Plate 78, F.

The full-grown larva (Pl. 76, U; fig. 16) is less translucent and the color is more grayish white than in the previous instar. There is a darker area almost the full length of the larva due to the contents of the digestive tract. The general shape of the larva is the same as in the previous instar. There is a distinct lateral invagination of the last abdominal segment. The dorsal folds are more prominent than in the previous instar, and there are usually more of them. The setæ are more prominent than in any previous instar except the first. Four full-grown larvæ averaged 2.485 mm. in length and 0.735 mm. in greatest width. Head as in previous instar. Mandibles (Pl. 76, R) very stout and heavily chitinized. The labrum (Pl. 76, S) is a large, rectangular, fleshy piece that almost covers the mandibles. On the inner surface near the distal margin are from 5 to 7 prominent denticles or lobes which are apparently very heavily chitinized; the labrum also bears 2 minute setæ. The maxillæ and labium (Pl. 76, T) are fused and only very slightly chitinized on the surface; they bear 6 minute setæ and in addition 2 groups of slight

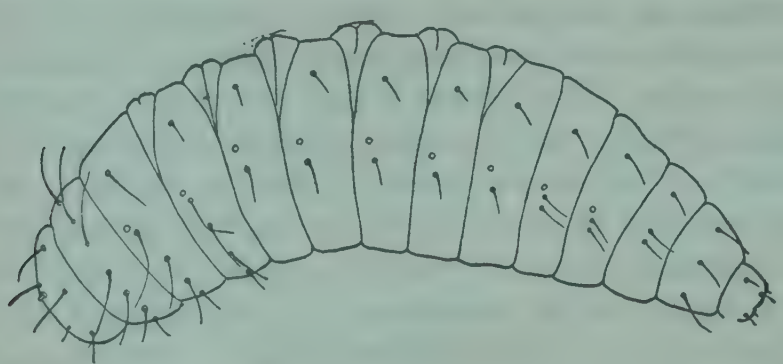


FIG. 16.—*Eupelmus allynii;* Lateral view of full-grown larva. × 30.6.

elevations or tubercles containing 3 elevations each, 2 groups containing 2 each, and 4 single scars or elevations. All are arranged as in Plate 76, T. Spiracles as in previous instar. Setæ as follows: 14 on the first thoracic, 10 on each of the second and third thoracic segments, 4 on each of the first 5 abdominal, 6 on each of the last 5 abdominal segments. Setæ arranged as shown in figure 16.

The number of larval instars and molts were ascertained in the manner described for *Ditropinotus aureoviridis*, page 411–412. There were found to be 4 larval molts with 5 pairs of larval mandibles (Pl. 76, N–R) as for *D. aureoviridis*. The manner of feeding and locomotion is also similar to that of *D. aureoviridis*. Thirty-two larvæ reared in glass cells in 1918 became full-grown in from 7 to 12 days, depending upon weather conditions. The average length of this period was 9 days, or an average of about 2.25 days per molt. Twenty-seven of these larvæ were inactive for from 2 to 17 days, an average of 5 days, before beginning pupation. In 1917, 28 individuals averaged 16 days in the larval stage from egg to prepupa.

This species proved to be much more tractable than either of the other two treated in this paper. They bred freely in the cell slides through five generations during the breeding season of 1918. The larvæ are very active and are voracious feeders. Never more than one individual was found to develop upon a single host larva, though as many as 8 eggs have been found in a single jointworm cell that was taken from a breeding cage. Under field conditions, however, this would scarcely obtain, since the jointworm cells so far outnumber the adults of *Eupelmus allynii* which are present.

PREPUPA

The prepupal stage (Pl. 78, G; 77, G) for 17 individuals of this species ran from 1 to 3 days, the average being 1.7 days. Upon entering this stage the larva voids considerable excrement, the prepupa being then pure white in color. At no other time has the larva ever been observed to void excrement. Upon entering this stage the larva contracts both in length and width. The third thoracic and first, second, and third abdominal segments become much shorter. There are numerous lateral fleshy folds at this time. The setæ are arranged the same as are those of the full-grown larva.

PUPA

When the pupa (Pl. 77, H) is first formed it is pure white. It gradually changes to intense black. Larvæ of this species that became full-grown in glass cells late in the fall usually did not pupate until the following spring, although a few remained in the pupal stage through the winter when kept in a cold cellar. Sixty-two individuals remained in the pupal stage from 7 to 33 days during the breeding season, depending upon weather conditions. The average length of the pupal period was 21 days. The pupa of the male is smaller than that of the female; the average length of 5 male pupæ was 2.142 mm. and the greatest width was 0.658 mm. The average length of 4 female pupæ was 3.0275 mm., and width 0.7875 mm.

ADULT

This species (fig. 13; 14; 15, *a*, *b*) overwinters as full-grown larvæ in the jointworm cells in old wheat stubble, the first adults emerging the latter part of April or the first week in May, in Virginia. Males normally occur, though under cage conditions this species will reproduce parthenogenetically, unfertilized females producing male progeny only.

The adults of this species are very active, and soon after emergence the females run rapidly up and down the old wheat stubbles nervously stroking the stubble with their antennæ in a diligent search for a suitable place to oviposit. They fly very quickly and rapidly but apparently only a few feet at a time. The manner of oviposition is

practically the same as that of *Ditropinotus aureoviridis* (see p. 414–415). The process of oviposition usually requires several minutes. One female *Eupelmus allynii* lived 52 days and oviposited over a period of 32 days. Four generations were reared in glass cell slides during the breeding season of 1918, starting with females that emerged from material collected the previous fall and kept under as nearly normal conditions as possible.

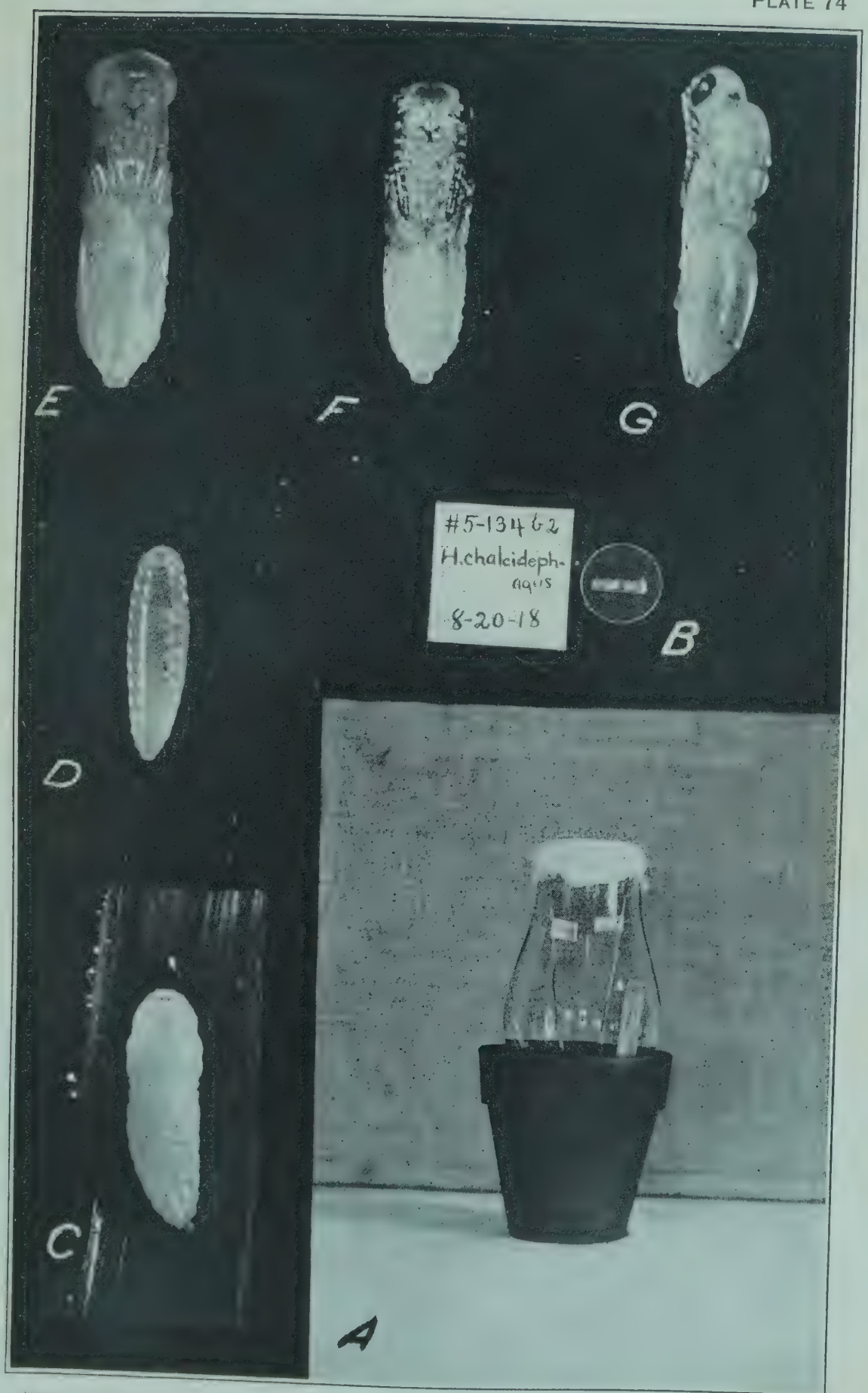
CONCLUSIONS

Judging from the observations recorded herewith and from the field observations of the season of 1919, it is quite clear why the parasites do not quickly gain complete control of the wheat jointworm. The writers have found that as the parasites become more abundant hyperparasitism increases greatly. Some of the parasites that are primary only, when present in small numbers, become both primary and secondary as they become more abundant.

At the close of the season in 1919 at Warrenton, Va., *Homoporus chalcidiphagus*, a purely primary parasite, was very scarce indeed, while in 1916 it was one of the most important. This condition is undoubtedly due to other parasites existing upon it as secondaries. *Eurytoma* sp. was the most important parasite present at Warrenton in the fall of 1919 and *Ditropinotus aureoviridis* and *Eupelmus allynii* were turning their attention largely to this species, since *Harmolita tritici* was greatly in the minority. While *Harmolita tritici* has been greatly in the minority in the fall in proportion to the number of its parasites present, for several years at Warrenton, the percentage of jointworm-infested wheat plants has remained about constant from year to year. This is further evidence that hyperparasitism has been going on continuously. Therefore it would seem that the parasites can be relied upon for only partial and not complete control.

PLATE 74

A.—Lantern globe breeding cage.
B.—Glass cell slide observation cage.
C.—Ventral view of the prepupa of *Homoporus chalcidiphagus*. × 13.
D.—Ventral view of full-grown larva of *H. chalcidiphagus*. × 9.4.
E.—Ventral view of female pupa of *H. chalcidiphagus*. × 12.9.
F.—Ventral view of male pupa of *H. chalcidiphagus*. × 14.5.
G.—Lateral view of pupa of *H. chalcidiphagus*. × 12.9.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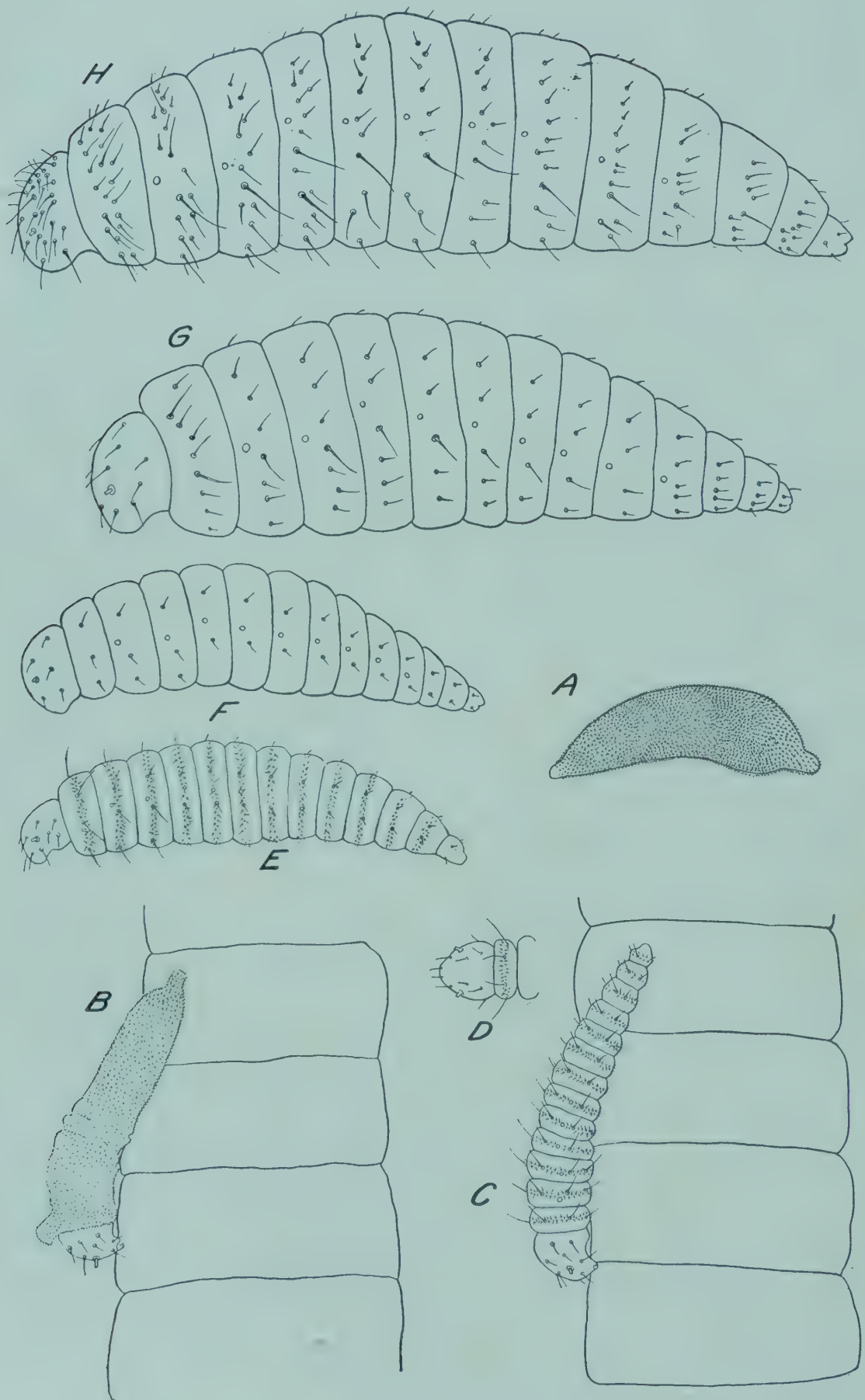
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PLATE 75

A.—Egg of *Ditropinotus aureoviridis*.

B.—Lateral view of *D. aureoviridis* larva hatching.

C.—Lateral view of *D. aureoviridis* larva just after hatching. Note the comparative size of larva and host.

D.—Dorsal view of head of first-instar larva of *D. aureoviridis*.

E.—Lateral view of first-instar larva of *D. aureoviridis* just before casting first molt.

F.—Lateral view of second-instar larva of *D. aureoviridis*.

G.—Lateral view of third-instar larva of *D. aureoviridis*.

H.—Lateral view of fourth-instar larva of *D. aureoviridis*.

All greatly enlarged.

42270°—21——5

PLATE 76

A.—Mandibles of first-instar larva of *Ditropinotus aureoviridis.*
B.—Mandibles of second-instar larva of *D. aureoviridis.*
C.—Mandibles of third-instar larva of *D. aureoviridis.*
D.—Mandibles of fourth-instar larva of *D. aureoviridis.*
E.—Mandibles of full-grown larva of *D. aureoviridis.*
F.—Mouthparts of full-grown larva of *D. aureoviridis.*
G.—First-instar mandibles of *Homoporus chalcidiphagus.*
H.—Second-instar mandibles of *H. chalcidiphagus.*
I.—Third-instar mandibles of *H. chalcidiphagus.*
J.—Fourth-instar mandibles of *H. chalcidiphagus.*
K.—Mandibles of full-grown larva of *H. chalcidiphagus.*
L.—Mouthparts of full-grown larva of *H. chalcidiphagus.*
M.—Ventral view of head of full-grown larva of *H. chalcidiphagus.*
N.—Mandibles of first-instar larva of *Eupelmus allynii.*
O.—Mandibles of second-instar larva of *E. allynii.*
P.—Mandibles of third-instar larva of *E. allynii.*
Q.—Mandibles of fourth-instar larva of *E. allynii.*
R.—Mandibles of full-grown larva of *E. allynii.*
S.—Labrum of full-grown larva of *E. allynii.*
T.—Mouthparts of full-grown larva of *E. allynii.*
U.—Lateral view of head of full-grown larva of *E. allynii.*
All greatly enlarged.

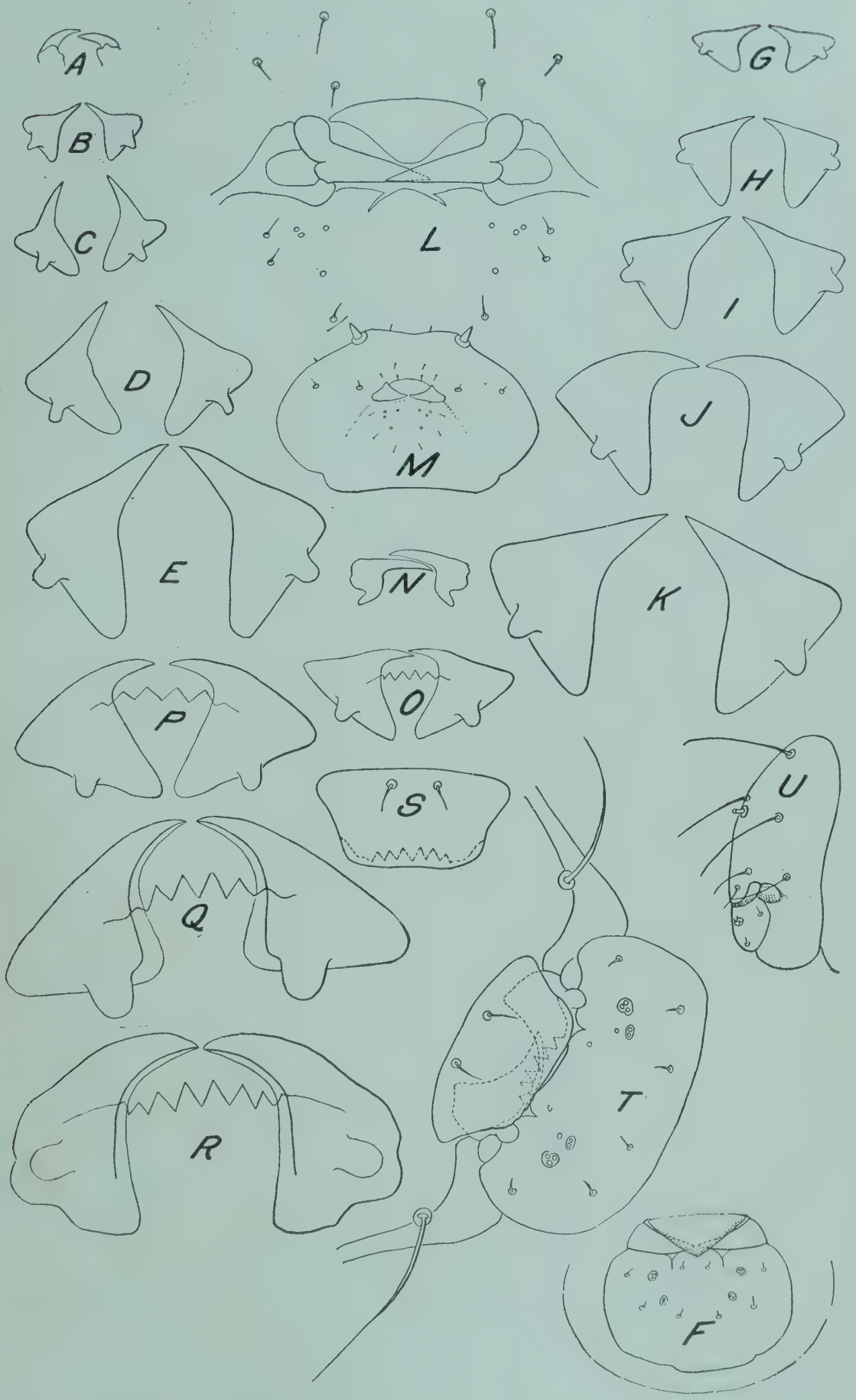
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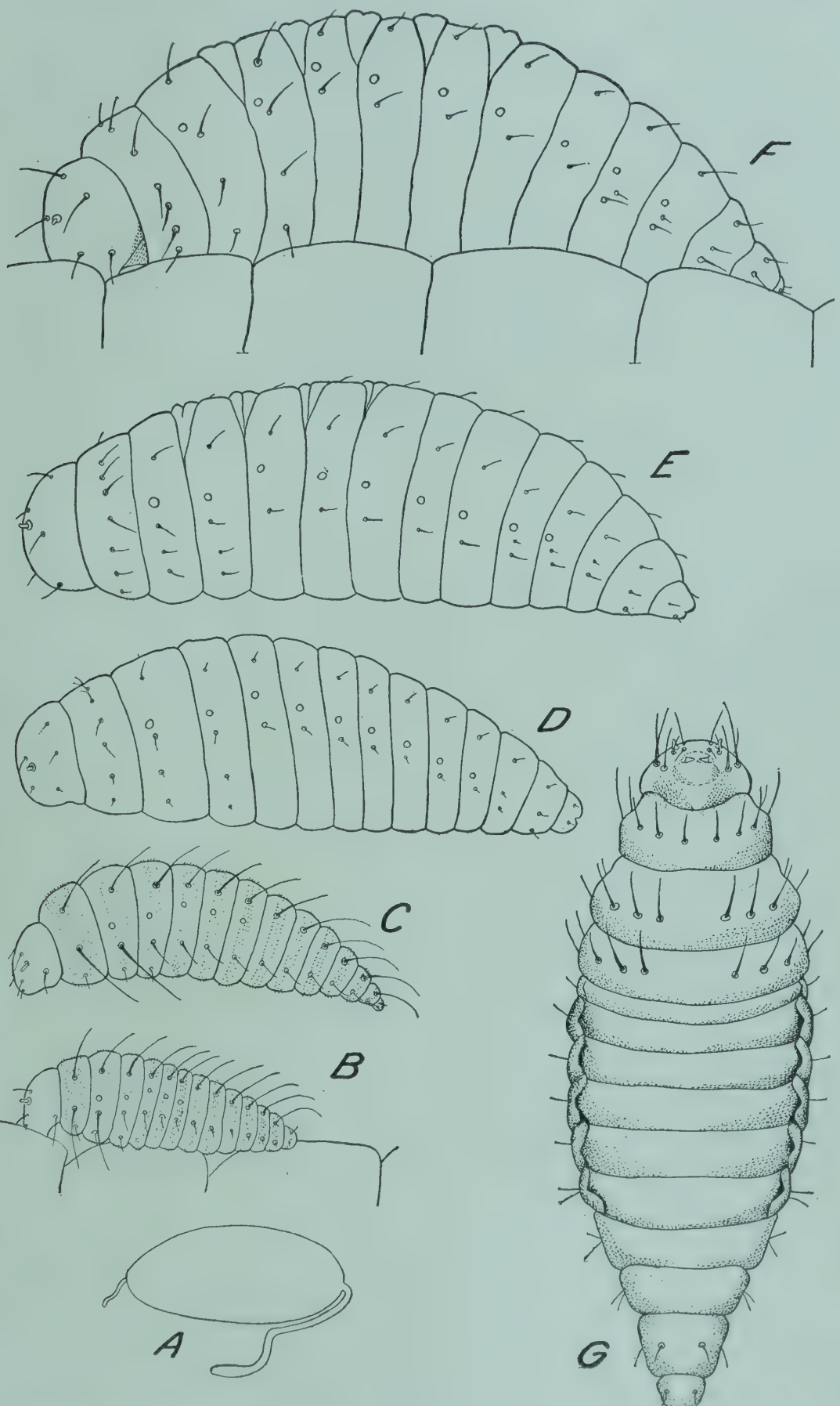
PLATE 77

A.—Ventral view of prepupal stage of *Ditropinotus aureoviridis*. × 13.7.
B.—Egg of *D. aureoviridis*. × 17.
C.—Lateral view of pupa of female *D. aureoviridis*. × 12.6.
D.—Lateral view of pupa of male *D. aureoviridis*. × 12.7.
E.—Egg of *Eupelmus allynii* in situ, showing netlike covering. × 19.4.
F.—Egg of *E. allynii* showing egg exposed and resting on netlike covering. × 19.4.
G.—Ventral view of prepupa of *E. allynii*. Greatly enlarged.
H.—Ventral view of pupa of *E. allynii*. × 12.2.

PLATE 78

A.—Egg of *Eupelmus allynii*. × 70.5.
B.—Lateral view of newly hatched *E. allynii* larva. × 86.7.
C.—Lateral view of first-instar larva of *E. allynii* just before casting first molt. × 86.7.
D.—Second-instar larva of *E. allynii*, lateral view.
E.—Third-instar larva of *E. allynii*, lateral view.
F.—Fourth-instar larva of *E. allynii*, lateral view.
G.—Ventral view of prepupa of *E. allynii*.
D to G greatly enlarged.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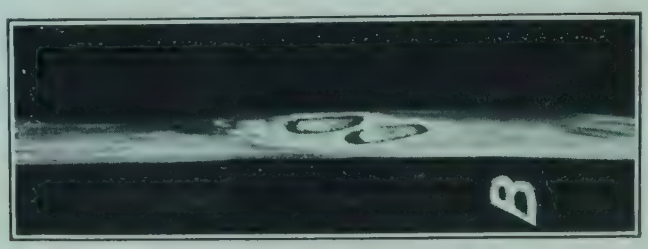
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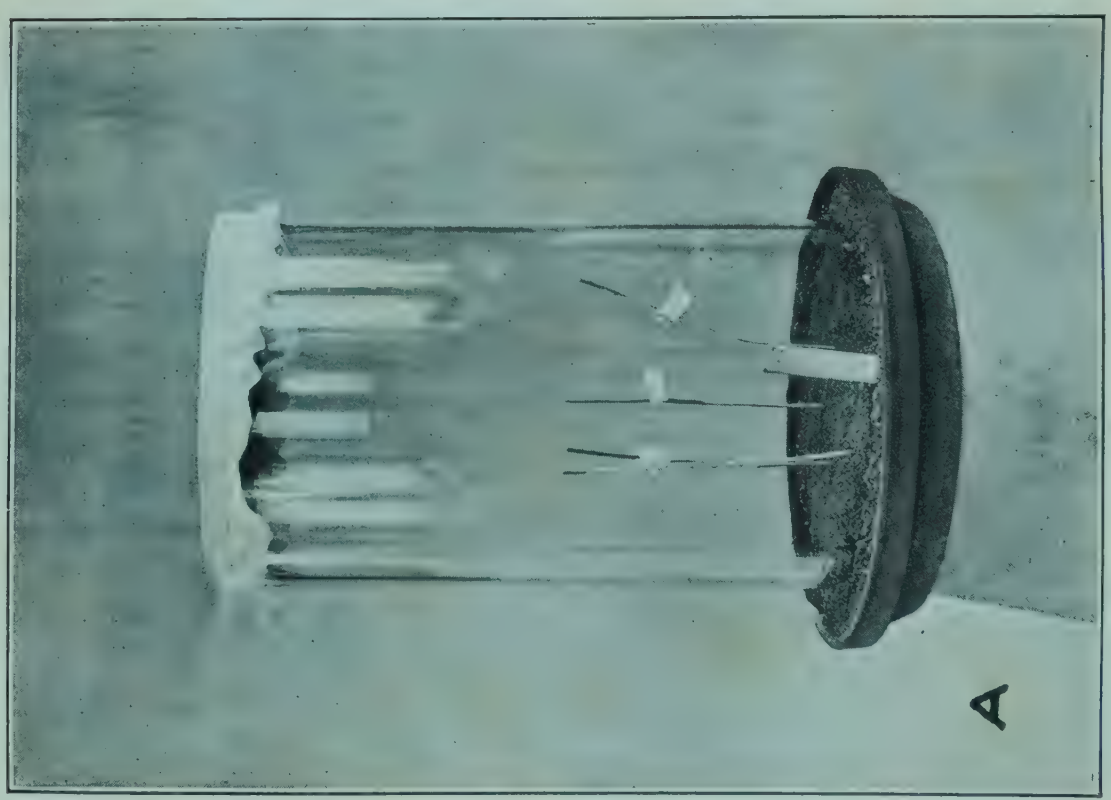
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PLATE 79

A.—Glass cylinder breeding cage, 9 by 14 inches.

B.—Two individual cells of a gall caused by the wheat jointworm in the culm, or stem, of the wheat.

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STUDY OF THE RELATION OF THE LENGTH OF KERNEL TO THE YIELD OF CORN. (ZEA MAYS INDENTATA).[1]

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Among corn growers there is a prevalent opinion that length or depth of kernel is a very desirable character in corn (*Zea mays indentata*) and that short kernels indicate deterioration. Rough, dented kernels are usually long and, therefore, this type is given preference by most growers in selecting seed corn, while smoothly dented ears are avoided.

Apparently, the relation of the length of kernel to the yield of corn has not been investigated directly. Some data on this subject, however, have been secured indirectly.

Montgomery [2] compared smooth and rough types of Reid Yellow Dent continuously selected for five years. Comparative yield data were secured for three seasons. The smooth-dented type outyielded the rough-dented type two out of three seasons and averaged 4.4 bushels more per acre. Although no data were presented to show the relative length of the kernels for the two lots of corn, it is probably safe to assume that those of the rough-dented type were the longer because of the correlation between indentation and length of kernels.

The writer [3] reported that smooth- or wrinkle-dented ears outyielded rough ears in ear-to-row tests of several varieties of corn conducted at the Kansas Agricultural Experiment Station.

Williams [4] compared smooth- and rough-dented strains of Clarage corn continuously selected for six years at the Ohio Agricultural Experiment Station and secured an average difference of 1.76 bushels per acre in favor of the smooth type.

Hutcheson and Wolfe [5] compared characters of a group each of high-yielding and low-yielding ears of Boone County White grown in ear-to-row

[1] Contribution No. 19 from Department of Agronomy, Agricultural Experiment Station of Kansas State Agricultural College.

[2] MONTGOMERY, E. G. EXPERIMENTS WITH CORN. Nebr. Agr. Exp. Sta. Bul. 112, p. 23. 1909.

[3] CUNNINGHAM, C. C. THE RELATION OF EAR CHARACTERS OF CORN TO YIELD. *In* Jour. Amer. Soc. Agron., v. 8, no. 3, p. 193. 1916.

[4] WILLIAMS, C. G., and WELTON, F. A. CORN EXPERIMENTS. Ohio Agr. Exp. Sta. Bul. 282, p. 87. 1915

[5] HUTCHESON, T. B., and WOLFE, T. K. RELATION BETWEEN YIELD AND EAR CHARACTERS IN CORN. *In* Jour. Amer. Soc. Agron., v. 10, no. 6, p. 253. 1918.

tests for two seasons. The length of kernel was one of the characters studied. The average length of the kernels in the high-yielding and low-yielding lots were 0.42 and 0.41 inches, respectively, and the yields were 72.5 and 54.8 bushels per acre, respectively. Concerning this point the authors conclude that length of the kernels was a negligible factor in the yields.

Olson, Bull, and Hayes [1] found that smooth and medium smooth ears of Minnesota No. 13 corn slightly outyielded rough ears in ear-to-row tests conducted at the Minnesota Agricultural Experiment Station.

PLAN OF THE EXPERIMENT

An experimental project was put under way at the Kansas Agricultural Experiment Station in 1916 to secure some data concerning the relation of length of kernel to the yield of corn. Commercial White, a medium large variety well adapted to growing on productive soils in eastern Kansas, was used. Three 40-ear lots of corn were selected. One lot was made up of ears with kernels that were relatively short, one of ears with kernels of maximum length, and the third of ears with kernels of medium length. The latter group was practically intermediate between the short- and long-kerneled ones. Since the degree of indentation is usually correlated with length of kernel, this character was taken as an index by which to classify the various ears. Only ears that were almost smooth or "dimple-dented" were selected for the short-kerneled group, while only ears that were sufficiently indented to have kernels with a chaffy crown or with at least an indication of chaffiness at the crown were used for the rough- or deep-kerneled group. In this latter group the endosperm as a rule was not completely filled. The kernels of the intermediate group were "wrinkle-dented," that is, the indentation was sufficient to cause a wrinkling of the epidermis of the kernel over the indented portion. The ears shown in Plate 81 are typical for the group, while Plates 80 and 82 show typical ears of the long- and short-kerneled groups, respectively. These groups are designated as "rough" and "smooth," respectively, while the group with kernels intermediate in length is known as "medium." This last group was practically identical in type and length of kernels with the mean for the Commercial White variety. Kernels of the three types are shown in Plate 83.

The seed for the various groups or types of ears was continuously selected. Smooth ears were selected each season from the progeny of smooth ears, rough ears from the progeny of rough ears, and medium ears from the progeny of medium ears.

In selecting the seed ears, care was taken to secure, if possible, ears that would conform to what is ordinarily considered the standard in seed corn. The ears were well-developed, with uniform kernels and in good seed condition.

[1] OLSON, P. J., BULL, C. P., and HAYES, H. K. EAR TYPE SELECTION AND YIELD IN CORN. Minn. Agr. Exp. Sta. Bul. 174, p. 28. 1918.

Determinations of yield were made in plots consisting of six to eight rows. They were planted in triplicate. The four inside rows only of each plot were harvested for yield. The corn was planted thick in 42-inch drill rows and thinned to a uniform stand of one stalk every 21 inches. No effort was made to control cross pollination between corn of the various types, and undoubtedly the usual amount of cross-fertilization took place.

The yields which are given in Table I are for shelled corn well dried in a heated room where the temperature varied from 60° to 80° F. The probable error was determined by using the formula $p.\ e. = 0.8453 = \frac{1}{n\sqrt{n-1}}$, in which n equals the number of replications.

TABLE I.—*Yields of smooth-, medium-, and rough-dented types of commercial white corn, 1916 to 1919*[1]

Type.	1916	1917	1918	1919	Four-year average.
Smooth	41.7±0.79	41.1±0.50	28.1±0.97	35.2±0.89	36.5±0.78
Medium	41.2± .33	43.9±1.29	23.0± .48	31.1± .35	34.8± .61
Rough	42.1± .86	45.3±1.09	20.3± .14	34.2± .89	35.5± .74

[1] Expressed in bushels per acre of shelled corn.

It will be noted that the smooth-dented type produced the highest 4-year average yield, and that the medium-dented type yielded the least. The data are not very consistent for the 4-year period. There was practically no difference in the yields the first season. In 1917 the rough type produced about 2 bushels more than the medium and about 4 bushels more than the smooth type. This variation appeared to be due entirely to peculiar seasonal conditions at the time the corn was beginning to pollinate. Drought and hot winds prevailed during the last of July and first week in August and greatly damaged corn that flowered during that time. Rains occurred frequently after August 7, making conditions very favorable for corn for the remainder of the season. The smooth type of corn started pollinating two or three days earlier than the other types, and the earliest plants were injured to some extent. Many of the ears were poorly pollinated, especially at the butt. The ears of the medium and smooth type appeared to have been normally pollinated. Since the rough type of corn was slightly the latest of the three types to flower, it more nearly escaped the detrimental effect of the drought and for that reason produced a larger yield.

In 1918 hot, dry weather prevailed throughout the fruiting period of the corn. Under these conditions the smooth type, because of its earlier maturity, had the advantage and produced about 5 bushels more than the medium type and about 8 bushels more than the rough type. The difference in the yields for that season are, no doubt, significant.

In 1919 hot, dry weather prevailed during July, and although the temperature was favorable during August the corn did not make a maximum development because of a deficiency of moisture. The corn was planted much too thick and unfortunately was not thinned until it was 3 or 4 feet high. This no doubt decreased the yields to a considerable extent. The smooth type sustained the greatest injury because of the relatively thick stand for that type. In spite of this handicap the smooth type made the highest yield, although the difference as compared with the rough type is probably not significant. Altogether, the results show that for the conditions of this test a smooth type of corn will yield fully as well as medium or rough types.

The effect of continuous selection for types on the length, circumference, and weight of the ears, number of rows of kernels per ear, the length of kernel, and the percentage of corn to cob were also studied. Data for these characters for the seed ears used for planting the respective type lots each season and for the progeny of each lot were recorded and tabulated.

LENGTH OF EARS

The effect on the length of ears is shown in Tables II and III. It will be noted that the smooth type of ear used for planting consistently averaged longer than those for the other types, and the average length of ear of the progeny of this type was slightly greater. On the other hand, the rough ears were shorter than the others. This same relation between indentation and length of ears is found in the progeny, as shown in Table III.

TABLE II.—*Frequency distribution of ears of various lengths used in planting the type test of Commercial White corn, 1916–1920*

Year and type.	Number of ears having a length (in inches) of—																					Number of ears.	Mean length.
	6	6¼	6½	6¾	7	7¼	7½	7¾	8	8¼	8½	8¾	9	9¼	9½	9¾	10	10¼	10½	10¾	11	11¼		
																							Inches.
1916:																							
Smooth	..	..	..	..	..	..	..	..	..	..	5	5	4	10	3	4	5	2	1	1	..	..	40	9.35
Medium	..	..	..	..	..	..	..	..	..	..	3	5	3	9	8	4	1	5	1	1	..	..	40	9.42
Rough	..	..	..	..	..	..	..	..	..	..	2	5	6	6	3	6	6	2	4	..	..	..	40	9.49
1917:																							
Smooth	..	..	..	..	..	..	3	..	3	4	4	4	4	2	2	1	5	3	2	..	1	1	40	9.16
Medium	..	..	..	..	..	..	..	..	2	1	6	7	8	3	3	4	2	2	2	..	..	..	40	9.13
Rough	..	..	..	..	..	..	1	1	1	1	6	5	8	4	3	2	4	2	1	..	..	1	40	9.14
1918:																							
Smooth	..	..	..	..	..	..	..	..	1	..	..	2	2	3	4	4	4	..	4	2	..	..	26	9.64
Medium	..	..	..	..	..	..	1	..	1	2	6	6	4	3	3	1	2	2	..	1	1	..	33	8.72
Rough	..	..	..	2	..	1	3	1	2	6	..	1	2	2	..	2	3	2	..	..	..	1	28	8.64
1919:																							
Smooth	..	..	..	..	..	1	1	2	2	3	4	3	2	1	..	..	1	..	..	..	..	..	20	8.42
Medium	..	..	..	..	..	1	2	4	2	2	2	2	2	2	..	..	..	..	..	..	..	..	20	7.83
Rough	2	1	1	1	2	1	..	..	2	2	3	2	..	..	1	..	1	..	..	..	..	..	20	7.01
1920:																							
Smooth	..	..	..	..	..	..	..	2	2	4	5	7	4	6	3	3	1	..	2	1	..	..	40	8.96
Medium	..	..	..	..	..	..	3	4	7	5	8	6	4	2	..	1	..	..	..	..	..	..	40	8.44
Rough	1	..	1	2	2	4	9	6	5	4	2	2	..	1	1	..	..	..	..	..	..	..	40	7.72
Five-year average:																							
Smooth	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	.....	9.11
Medium	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	.....	8.72
Rough	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	.....	8.40

TABLE III.—*Frequency distribution of ears of various lengths in the progeny of smooth-, medium-, and rough-dented types of Commercial White corn, 1916 to 1919*

Year and type.	Number of ears having a length (in inches) of—																					Number of ears.	Mean length.
	2	2½	3	3½	4	4½	5	5½	6	6½	7	7½	8	8½	9	9½	10	10½	11	11½	12		
1916:																							*Inches.*
Smooth	...	...	3	5	5	8	13	19	24	23	38	39	56	70	67	62	42	22	6	2	...	504	8.09
Medium	...	...	10	2	4	9	14	13	23	25	32	41	51	71	66	46	34	11	6	1	...	459	8.07
Rough	...	2	5	3	7	7	11	14	20	18	27	53	49	76	80	42	38	15	1	1	...	469	8.02
1917:																							
Smooth	...	...	3	9	9	18	21	16	18	27	31	28	43	61	66	62	39	19	17	1	2	490	8.14
Medium	...	...	1	4	8	15	12	20	21	23	25	39	54	67	61	66	41	34	11	2	2	506	8.19
Rough	...	...	3	6	10	14	14	24	27	22	36	43	56	63	58	75	37	19	13	10	1	530	8.05
1918:																							
Smooth	...	3	4	11	13	20	28	29	53	79	107	96	94	57	28	12	4	1	...	.....	...	639	6.95
Medium	1	5	4	14	13	36	31	53	80	81	104	67	46	21	8	1	...	.....	.....	.....	...	565	6.39
Rough	...	5	10	8	12	20	31	31	73	69	64	81	53	18	7	4	...	2	...	.....	...	488	6.53
1919:																							
Smooth	1	1	2	2	10	8	13	23	29	46	61	99	121	129	88	45	19	6	2	.....	...	705	7.77
Medium	...	...	2	2	7	14	13	21	35	48	72	114	128	92	60	25	14	2	...	.....	...	649	7.57
Rough	...	1	5	9	6	13	20	32	56	67	64	121	107	109	52	23	6	2	...	.....	...	692	7.33
Four-year average:																							
Smooth	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.....	...	.....	7.74
Medium	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.....	...	.....	7.56
Rough	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.....	...	.....	7.48

CIRCUMFERENCE OF EARS

As may be seen in Table IV, which shows the circumferences of smooth, medium, and rough types, the rough ears are relatively large in circumference, those of the medium type are intermediate, and the smooth ears are small. The differences are consistent and probably significant. Similar differences for the progeny of these ears occur as shown in Table V.

TABLE IV.—*Frequency distribution of ears of various circumferences used for planting type test of Commercial White corn, 1916–1920*

Year and type.	Number of ears having a circumference (in inches) of—																					Number of ears.	Average circumference.
	5	5⅛	5¼	5⅜	5½	5⅝	5¾	5⅞	6	6⅛	6¼	6⅜	6½	6⅝	6¾	6⅞	7	7⅛	7¼	7⅜	7½	7⅝	
1916:																							*Inches.*
Smooth	...	...	...	...	...	...	...	...	...	...	...	...	4	4	9	11	7	4	1	...	...	...	40	6.84
Medium	...	...	...	...	...	...	...	...	...	...	...	...	2	4	7	7	10	8	2	...	...	...	40	6.91
Rough	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4	2	8	6	7	10	3	...	40	7.16
1917:																							
Smooth	...	...	...	...	...	...	1	1	7	5	10	3	6	5	1	...	...	...	1	...	...	...	40	6.28
Medium	...	...	...	...	...	...	...	...	1	1	5	10	10	3	3	3	3	...	...	1	...	...	40	6.53
Rough	...	...	...	...	...	...	...	...	...	2	1	3	4	5	3	7	4	2	5	2	...	1	40	6.81
1918:																							
Smooth	...	...	...	...	...	...	3	1	8	2	4	3	3	...	2	...	...	...	...	...	...	...	26	6.19
Medium	...	...	...	...	...	...	...	...	2	...	5	4	5	3	5	5	1	1	1	...	...	1	33	6.60
Rough	...	...	...	...	...	...	...	...	...	1	2	...	3	1	5	2	8	1	3	1	...	1	28	7.21
1919:																							
Smooth	...	1	1	3	4	2	2	4	2	1	...	...	...	...	...	...	...	...	...	...	...	...	20	5.96
Medium	...	...	...	1	1	2	3	4	4	2	1	...	1	1	...	...	...	...	...	...	...	...	20	6.16
Rough	...	...	...	...	...	...	...	...	...	3	4	3	3	3	2	1	1	...	...	...	...	...	20	6.55
1920:																							
Smooth	...	...	...	...	4	9	8	13	4	2	...	...	...	...	...	...	...	...	...	...	...	...	40	6.06
Medium	...	...	...	...	...	1	2	7	19	8	2	1	...	...	...	...	...	...	...	...	...	...	40	6.51
Rough	...	...	...	...	...	...	...	...	4	7	15	8	4	1	1	...	...	...	...	...	...	...	40	7.05
Five-year average:																							
Smooth	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.....	6.27
Medium	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.....	6.54
Rough	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.....	6.96

TABLE V.—*Frequency distribution of ears of various circumferences in the progeny of smooth-, medium-, and rough-dented types of Commercial White corn, 1916–1919*

Year and type.	Number of ears having a circumference (in inches) of—																							Number of ears.	Average circumference.
	2¼	2½	2¾	3	3¼	3½	3¾	4	4¼	4½	4¾	5	5¼	5½	5¾	6	6¼	6½	6¾	7	7¼	7½	7¾	8	8¼		
1916:																											*Inches.*
Smooth	...	...	...	...	...	...	...	1	...	6	7	11	34	27	39	65	105	81	73	36	10	8	1	...	...	504	6.21
Medium	...	...	...	...	...	...	...	7	...	6	6	8	15	30	50	80	70	97	37	37	12	3	1	...	...	459	6.27
Rough	...	...	...	...	...	...	...	...	...	2	2	15	13	24	27	73	92	89	54	42	22	10	4	...	...	469	6.32
1917:																											
Smooth	...	...	...	...	...	1	1	1	...	4	5	16	19	25	54	74	77	93	62	31	16	8	2	1	...	490	6.22
Medium	...	...	...	...	...	...	...	...	...	3	1	20	12	22	29	44	63	93	82	63	38	27	6	3	...	506	6.44
Rough	...	...	...	...	...	1	1	1	2	4	1	9	15	17	28	39	46	101	84	79	48	30	7	15	2	530	6.57
1918:																											
Smooth	...	...	...	...	...	...	...	4	10	17	19	54	79	140	107	119	57	23	7	3	...	...	...	...	...	639	5.29
Medium	...	...	...	4	...	3	...	9	13	16	23	36	54	109	100	114	54	23	4	6	...	...	...	...	...	565	5.59
Rough	...	...	...	2	3	...	...	8	7	7	11	26	34	87	92	108	47	40	12	3	1	...	...	...	...	488	5.72
1919:																											
Smooth	1	1	...	3	...	1	1	10	9	12	17	38	49	93	106	156	91	78	26	11	...	...	2	...	...	705	5.79
Medium	...	...	...	1	1	3	2	3	1	7	11	26	34	99	66	132	92	103	38	22	4	2	2	...	...	649	5.94
Rough	...	1	2	...	1	2	1	4	4	8	6	20	19	56	47	90	104	143	75	72	27	12	8	1	...	692	6.21
Four-year average:																											
Smooth	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.....	5.88
Medium	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.....	6.06
Rough	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.....	6.21

WEIGHT OF EARS

The average weight of the seed ears of the three types used in planting varied but little after the first season. The data given in Table VI show that the smooth ears averaged slightly less than the medium, and the rough type slightly heavier. The differences are not entirely consistent and probably are not great enough to be considered significant. The data for the progeny as given in Table VII also show that there is but little difference in the average weight of the ears of the various lots.

TABLE VI.—*Frequency distribution of ears of various weights used in planting type test of Commercial White corn for 1916–1920*

Year and type.	Number of ears weighing (in grams)—											Number of ears.	Average weight.
	175 to 200.	200 to 225.	225 to 250.	250 to 275.	275 to 300.	300 to 325.	325 to 350.	350 to 375.	375 to 400.	400 to 425.	425 to 450.		
1916: [a]													*Gm.*
Smooth	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Medium	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Rough	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1917:													
Smooth	.....	.....	1	1	6	8	10	7	6	1	.....	40	321
Medium	.....	.....	.....	.....	1	9	15	11	3	1	.....	40	330
Rough	.....	.....	.....	3	3	3	9	10	7	3	2	40	361
1918:													
Smooth	.....	.....	1	4	7	7	3	3	1	.....	.....	26	306
Medium	.....	1	.....	3	5	12	5	4	2	1	.....	33	313
Rough	.....	2	2	5	7	2	3	4	3	.....	.....	28	300
1919:													
Smooth	.....	4	6	7	3	.....	.....	.....	.....	.....	.....	20	250
Medium	.....	4	7	4	2	1	1	1	.....	.....	.....	20	257
Rough	2	2	3	6	4	2	1	.....	.....	.....	.....	20	260
1920:													
Smooth	.....	.....	7	13	6	6	4	3	1	.....	.....	40	286
Medium	.....	1	3	6	11	11	6	2	.....	.....	.....	40	295
Rough	1	1	3	5	13	9	5	3	.....	.....	.....	40	295
Five-year average:													
Smooth	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	290.9
Medium	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	299.0
Rough	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	304.2

[a] No data.

TABLE VII.—*Frequency distribution of ears of various weights in the progeny of smooth-, medium-, and rough-dented types of corn, 1916–1919*

Year and type.	Number of ears weighing (in grams)—										Number of ears.	Average weight.
	0 to 50.	50 to 100.	100 to 150.	150 to 200.	200 to 250.	250 to 300.	300 to 350.	350 to 400.	400 to 450.	450 to 500.		
												Gm.
1916:												
Smooth	14	37	36	56	68	90	105	74	22	2	504	257.1
Medium	15	27	28	62	66	95	89	68	9	.....	459	252.6
Rough	4	37	31	37	72	106	107	54	19	2	469	261.5
1917:												
Smooth	10	40	59	62	87	106	72	39	15	.....	490	228.3
Medium	17	43	47	54	95	106	91	47	11	.....	506	233.5
Rough	16	43	48	67	90	92	113	42	19	.....	530	239.8
1918:												
Smooth	38	73	119	218	154	34	3	.....	.....	.....	639	163.4
Medium	59	86	125	155	119	19	4	.....	.....	.....	565	148.1
Rough	42	65	105	157	91	25	3	.....	.....	.....	488	153.4
1919:												
Smooth	23	57	91	120	182	173	49	7	.....	.....	705	202.4
Medium	15	41	80	138	191	130	51	3	.....	.....	649	206.5
Rough	22	55	79	135	136	160	89	14	2	.....	692	213.6
Four-year average:												
Smooth	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	210.3
Medium	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	209.9
Rough	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	217.9

NUMBER OF ROWS OF KERNELS PER EAR

The data for number of rows of kernels per ear for the corn used in planting the type test were not determined the first season. The results for 1917, 1918, 1919, and 1920 are given in Table VIII. The data show that the mean number of rows per ear has remained practically stationary in the medium type, decreased in the smooth type, and increased in the rough type. Data for the progeny show similar though somewhat smaller differences.

TABLE VIII.—*Frquency distribution of ears with various number of rows of kernels used for planting type test of Commercial White corn for 1917–1920*

Year and type.	Number of ears with—						Number of ears.	Average number of rows.
	12 rows.	14 rows.	16 rows.	18 rows.	20 rows.	22 rows.		
1917:								
Smooth	10	21	8	1	.....	.....	40	14.0
Medium	.....	17	19	3	1	.....	40	15.4
Rough	.....	11	20	7	2	.....	40	16.0
1918:								
Smooth	8	12	5	1	.....	.....	26	13.9
Medium	1	13	15	2	2	.....	33	15.5
Rough	.....	6	11	9	2	.....	28	16.5
1919:								
Smooth	4	13	2	1	.....	.....	20	14.0
Medium	1	7	10	2	.....	.....	20	15.3
Rough	.....	3	7	7	3	.....	20	17.0
1920:								
Smooth	8	25	5	2	.....	.....	40	14.1
Medium	3	13	20	4	.....	.....	40	15.3
Rough	.....	6	14	14	5	1	40	17.1
Four-year average:								
Smooth	.....	.....	.....	.....	.....	.....	.....	14.0
Medium	.....	.....	.....	.....	.....	.....	.....	15.4
Rough	.....	.....	.....	.....	.....	.....	.....	16.7

TABLE IX.—*Frequency distribution of ears with various numbers of rows of kernels in the progeny of smooth, medium, and rough types of corn, 1916–1919*

Year and type.	Number of ears with—											Number of ears.	Average number of rows.
	6 rows.	8 rows.	10 rows.	12 rows.	14 rows.	16 rows.	18 rows.	20 rows.	22 rows.	24 rows.	26 rows.		
1916:													
Smooth	.....	2	17	114	179	124	45	20	2	1	.....	504	14.5
Medium	.....	.....	9	83	152	142	55	17	1	.....	.....	459	14.9
Rough	.....	.....	5	66	171	138	70	14	3	1	1	469	15.1
1917:													
Smooth	.....	1	7	112	183	114	47	12	3	1	.....	490	14.2
Medium	.....	.....	14	69	170	157	71	18	6	1	.....	506	15.0
Rough	.....	.....	13	89	165	160	83	45	4	1	.....	530	15.0
1918:													
Smooth	.....	.....	27	155	253	150	44	9	1	.....	.....	639	13.9
Medium	.....	.....	25	109	209	155	57	11	1	.....	.....	565	14.5
Rough	.....	.....	20	81	181	143	50	9	2	.....	.....	488	14.7
1919:													
Smooth	3	11	27	147	295	171	44	6	1	.....	.....	705	14.1
Medium	1	6	21	98	230	208	70	14	1	.....	.....	649	14.7
Rough	4	4	11	81	206	219	119	38	8	1	1	692	15.4
Four-year average:													
Smooth	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	14.2
Medium	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	14.8
Rough	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	15.1

LENGTH OF KERNELS

The data presented in Table X show that the length of kernels of the ears selected for planting have averaged 0.422 inch for the smooth type, 0.473 for the medium, and 0.512 for the rough. Continuous selecting of smooth, medium, and rough ears appears not to have changed the relative length of the kernels during four generations, as shown in Table XI. In other words, there has been a marked regression toward the mean of the variety, which probably indicates that the length of the kernel is influenced more by environment than the other characters that have been studied.

TABLE X.—*Frequency distribution of ears with various lengths of kernels of Commercial White corn used in planting, 1916–1919*

Year and type.	Number of kernels having a length (in inches) of—								Number of ears.	Average length.
	11/32	12/32	13/32	14/32	15/32	16/32	17/32	18/32		
1916:										*Inches.*
Smooth	.....	4	19	17	.....	.....	.....	.....	40	0.416
Medium	.....	.....	.....	15	16	9	.....	.....	40	.478
Rough	.....	.....	.....	.....	.....	12	15	13	40	.532
1917:										
Smooth	1	10	15	10	2	2	.....	.....	40	.413
Medium	.....	1	1	5	12	17	3	1	40	.481
Rough	.....	.....	.....	4	10	19	4	3	40	.494
1918:										
Smooth	.....	2	8	8	6	2	.....	.....	26	.435
Medium	.....	.....	1	10	10	11	1	.....	33	.470
Rough	.....	.....	2	4	9	10	3	.....	28	.487
1919:										
Smooth	.....	2	6	8	4	.....	.....	.....	20	.428
Medium	.....	.....	3	2	12	2	.....	1	20	.464
Rough	.....	.....	.....	.....	1	12	2	5	20	.517
Four-year average:										
Smooth	.....	.....	.....	.....	.....	.....	.....	.....	.....	.422
Medium	.....	.....	.....	.....	.....	.....	.....	.....	.....	.473
Rough	.....	.....	.....	.....	.....	.....	.....	.....	.....	.512

TABLE XI.—*Frequency distribution of ears of various lengths of kernels in the progeny of smooth, medium, and rough corn, 1916–1919*

Year and type.	Number of kernels having a length (in inches) of—													Number of ears.	Average length of kernel.
	7/32	8/32	9/32	10/32	11/32	12/32	13/32	14/32	15/32	16/32	17/32	18/32	19/32		
1916:															*Inches.*
Smooth	1	3	2	27	39	139	143	110	28	11	1	.....	.....	504	0.383
Medium	....	7	5	12	32	97	115	116	34	36	4	1	.....	459	.407
Rough	....	1	3	17	27	70	105	128	48	56	6	5	3	469	.411
1917:															
Smooth	2	6	14	44	84	166	88	57	18	7	2	2	.....	490	.371
Medium	6	9	18	47	63	150	92	65	35	16	5	.....	.....	506	.385
Rough	9	17	26	50	81	155	86	62	22	17	5	.....	.....	530	.375
1918:															
Smooth	7	17	35	83	113	195	94	59	26	5	1	1	.....	639	.358
Medium	15	12	18	37	74	182	95	65	49	16	2	1	.....	565	.379
Rough	13	5	15	41	35	145	63	75	41	41	5	8	.....	488	.385
1919:															
Smooth	1	17	18	51	81	137	124	134	71	54	16	1	.....	705	.400
Medium	2	1	17	36	58	120	133	147	79	50	3	2	.....	649	.409
Rough	....	7	7	24	51	82	135	177	116	66	23	4	.....	692	.425
Four-year average:															
Smooth	....	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.378
Medium	....	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.395
Rough	....	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.399

TRANSMISSION OF INDENTATION IN THE PROGENY OF SMOOTH, MEDIUM, AND ROUGH TYPES OF CORN

The number of ears of each type in the progeny of each type is shown in Table XII. The results are illustrated in figure 1 and Plates 84 to 87. The data show that there was a marked tendency for the smooth, medium,

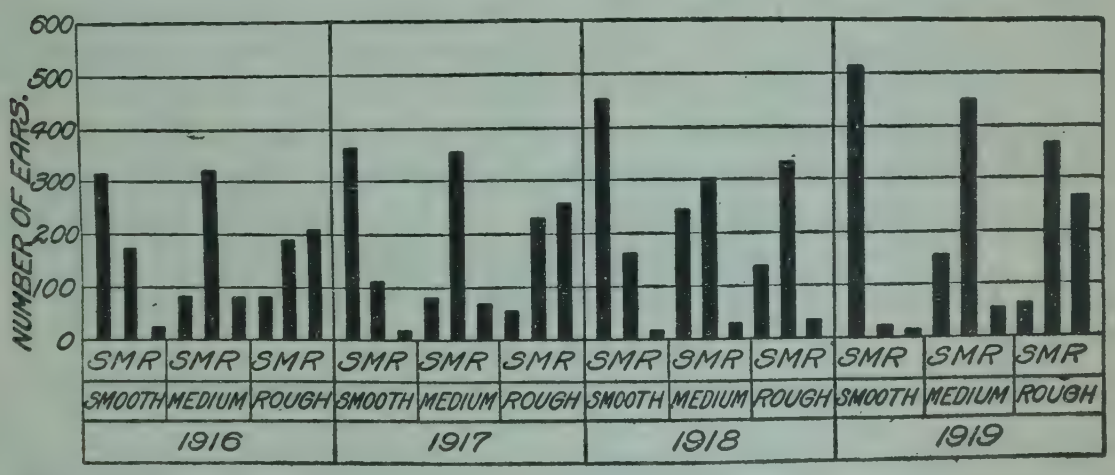


FIG. 1.—Transmission of the character of indentation in the progeny of continuously selected smooth, medium, and rough types of corn. The columns show the number of ears of the respective types in the progeny of smooth-, medium-, and rough-dented Commercial White corn for each season.

and rough types to transmit their respective type characters to the progeny each season. This fact is most apparent in the smooth type. For each successive season the proportion of corn of the smooth type in the progeny increased over that for the preceding season. There was very little variation in the medium type except for the dry season of 1918. The percentage of ears of the smooth type that season was greatly increased, apparently because of the effect of drought on the development of the ears. This also occurred in 1919, but not to the same extent.

The rough type failed to produce as high a percentage of ears of the parent type as did the others. This was especially true in the dry seasons of 1918 and 1919. It seems, therefore, that seasonal conditions, especially drought, have a decided effect on the indentation.

TABLE XII.—*Transmission of the character of indentation in the progeny of smooth, medium, and rough types of corn, 1916–1919*

Year and type.	Number of ears in the progeny.		
	Smooth.	Medium.	Rough.
1916:			
Smooth	315	72	75
Medium	172	316	190
Rough	17	71	204
1917:			
Smooth	364	83	48
Medium	114	356	226
Rough	12	67	246
1918:			
Smooth	454	241	130
Medium	160	301	325
Rough	9	25	32
1919:			
Smooth	515	152	61
Medium	186	450	368
Rough	4	47	263

RATIO OF GRAIN TO COB

The relation between smoothness of ears and the ratio of grain to cob are shown in Tables XIII and XIV. These data show quite conclusively that the percentage of grain to cob increases directly with the length of the kernel. For every season the smooth type had the lowest percentage of grain to cob in the ears used for planting and in the progeny, while the rough type had the highest percentage. The percentage of grain to cob varies with the seasons. It was relatively high in the season of 1917 when conditions were very favorable for corn during the fruiting period, and it was relatively low during the hot, dry season of 1918.

TABLE XIII.—*Ratio of grain to cob in smooth, medium, and rough ears used for planting 1916–1920*

Year and type.	Percentage of grain to cob.	Year and type.	Percentage of grain to cob.
1916:		1919:	
Smooth	77.9	Smooth	76.9
Medium	79.5	Medium	79.0
Rough	80.9	Rough	81.2
1917:		1920:	
Smooth	82.96	Smooth	80.1
Medium	83.70	Medium	82.3
Rough	83.93	Rough	84.0
1918:		Five-year average:	
Smooth	80.8	Smooth	79.7
Medium	81.6	Medium	81.2
Rough	82.4	Rough	82.5

TABLE XIV.—*Percentage of grain to cob for the progeny of smooth, medium, and rough ears, 1916–1919*

Year and type.	Percentage of grain to cob.	Year and type.	Percentage of grain to cob.
1916:		1919:	
Smooth	77.9	Smooth	76.9
Medium	79.5	Medium	79.0
Rough	80.5	Rough	81.2
1917:		Four-year average:	
Smooth	80.7	Smooth	78.2
Medium	81.4	Medium	79.7
Rough	82.3	Rough	81.0
1918:			
Smooth	77.5		
Medium	78.9		
Rough	80.0		

PRACTICAL APPLICATION

Because of the popular opinion that smoothness of ears is an indication of deterioration, the results of this study are of practical interest. Smooth corn has several advantages over medium or rough. Every corn grower knows that the smooth type is much to be preferred at husking time. This type is not so subject to damage from molds and other fungi following injury to the ears from the corn earworm. The latter factor is often an important one, since moldy corn is dangerous to feed. Rough ears, because of the length of kernel and circumference of the ears, do not dry out as rapidly as the smooth type, and for this reason they are more likely to be low in vitality as a result of freezing. Smooth kernels are less likely to rot when conditions for germination are unfavorable. It would seem, therefore, that the corn grower should not hesitate to select smooth ears. It may be well to select slightly rougher ears than are desired in the progeny, since there appears to be a decided tendency to vary toward the smooth type. It is also well to do this in order to avoid a hard, flinty type of kernel that would be unsatisfactory for feeding without grinding.

CONCLUSIONS

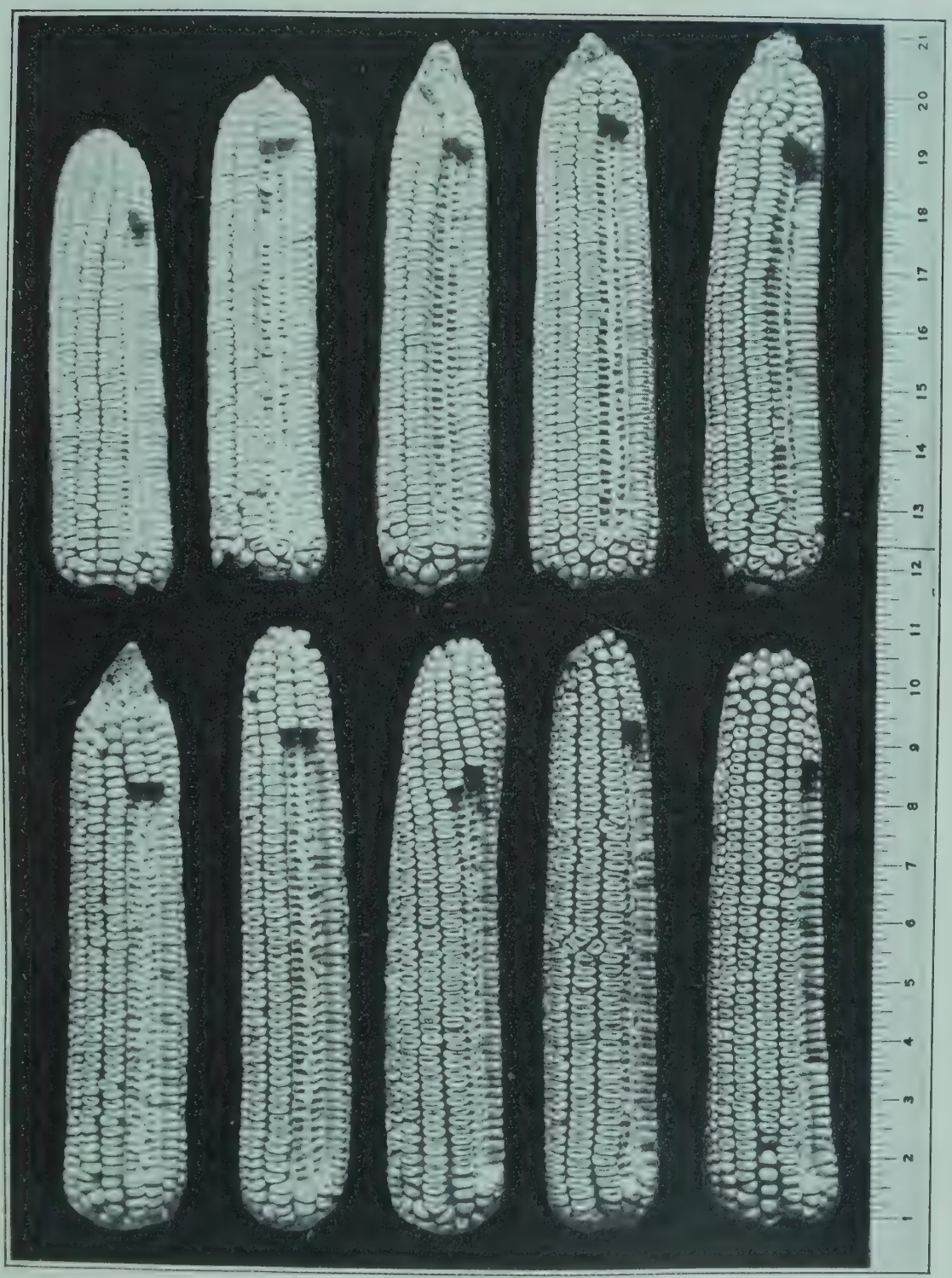
The popular opinion that smoothness in corn is an indication of deterioration and reduced yielding capacity appears to be erroneous. In the experiments reported the smooth type yielded as well as the medium and rough types on the average and indicated that under adverse conditions it will yield better.

Continuous selection of smooth and rather short kernels for four generations increased the average length of the ears, decreased the circumference, slightly decreased the weight, decreased the number of rows per ear, and decreased the length of the kernel and the percentage of shelled grain.

On the other hand, continuous selection of rough and rather long kernels decreased the average length of the ear and increased the circumference but had no significant effect on the weight of ears, the number of rows per ear, the length of the kernel, or the percentage of grain.

PLATE 80

Typical ears of the smooth-dented or short-kerneled type selected from Commercial White corn.



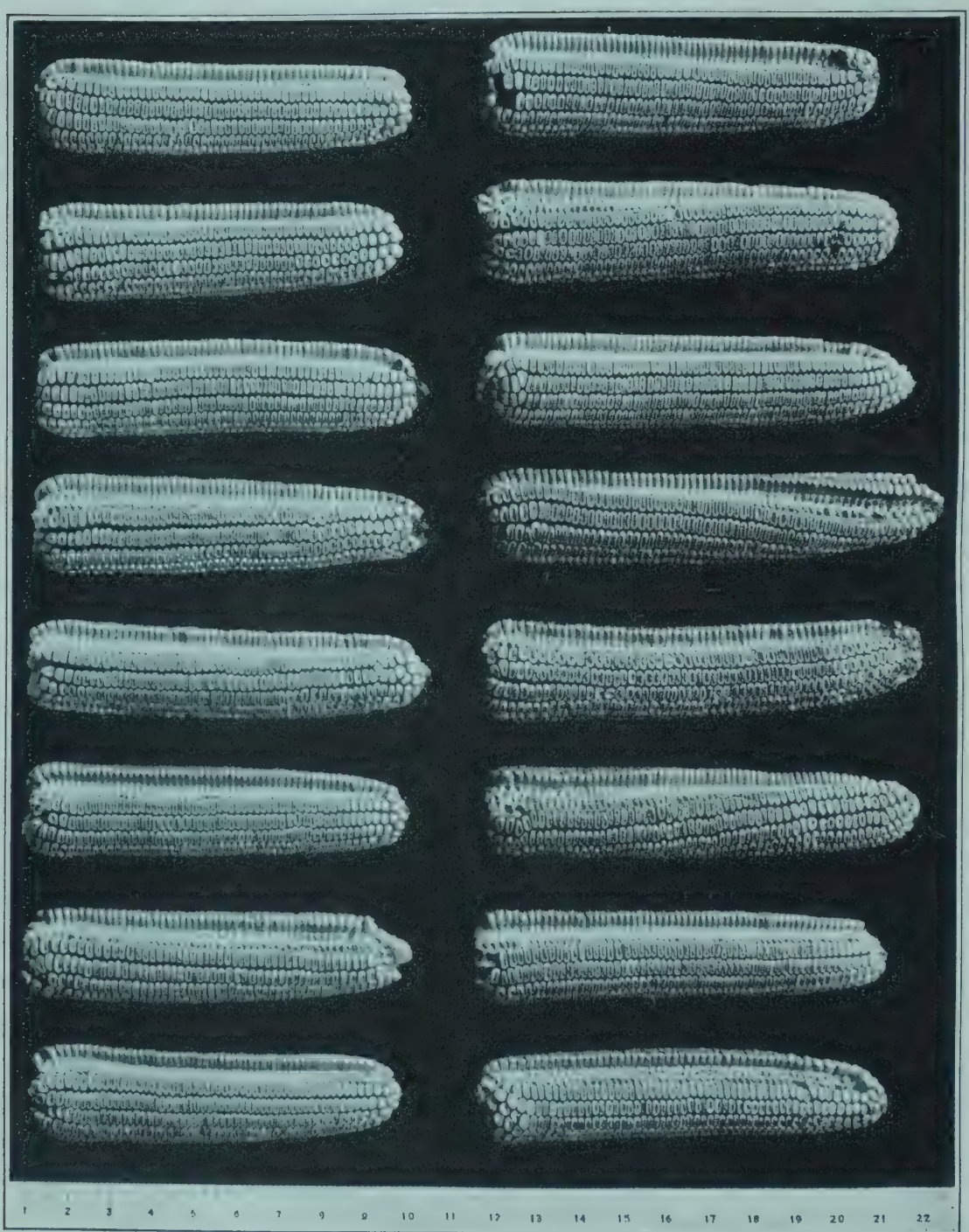
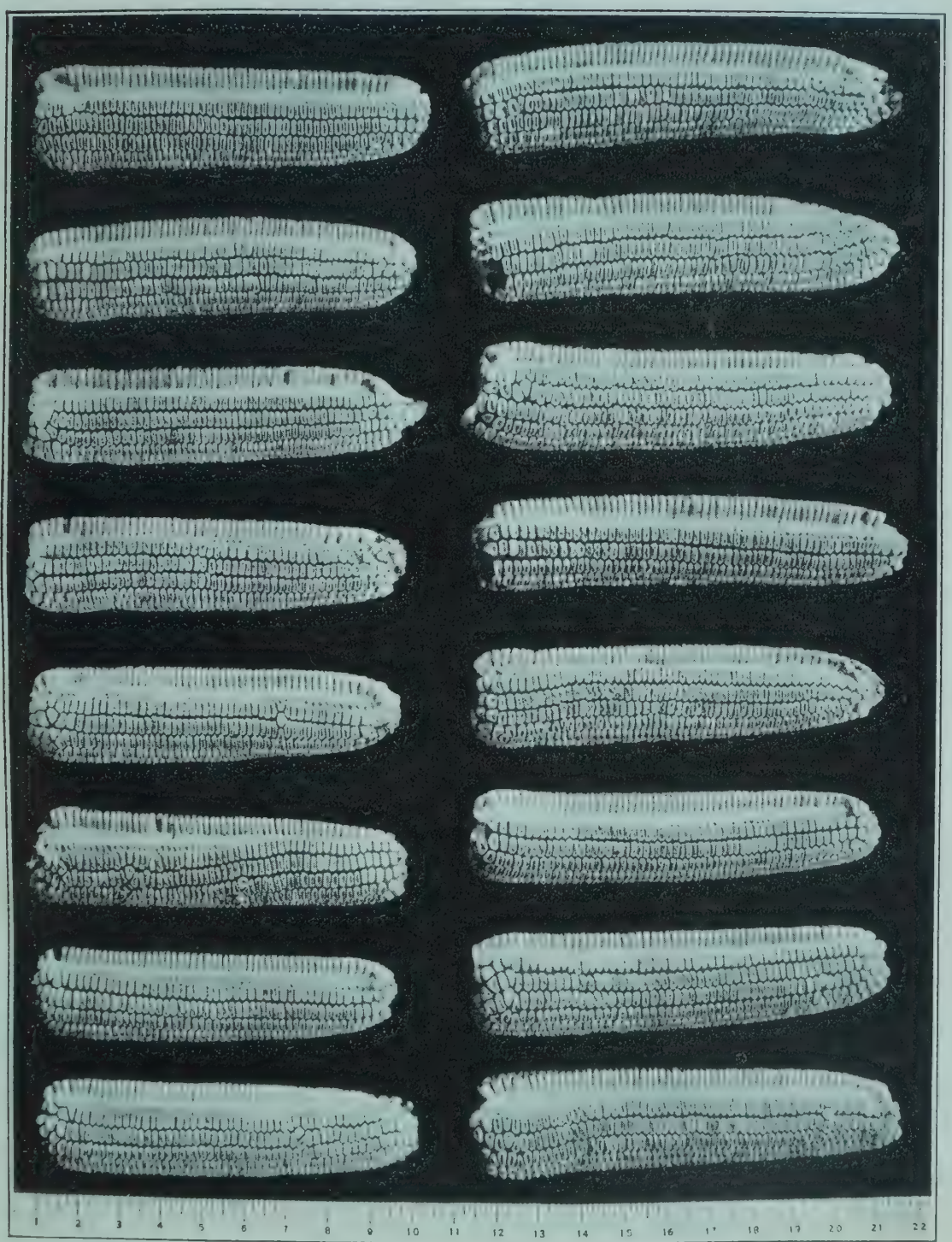

1 2 3 4 5 6 7 9 9 10 11 12 13 14 15 16 17 18 19 20 21 22

PLATE 81

Typical ears of the medium-dented or medium length of kernel type of Commercial White corn.

PLATE 82

Typical ears of the rough-dented or long-kerneled type of Commercial White corn.


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

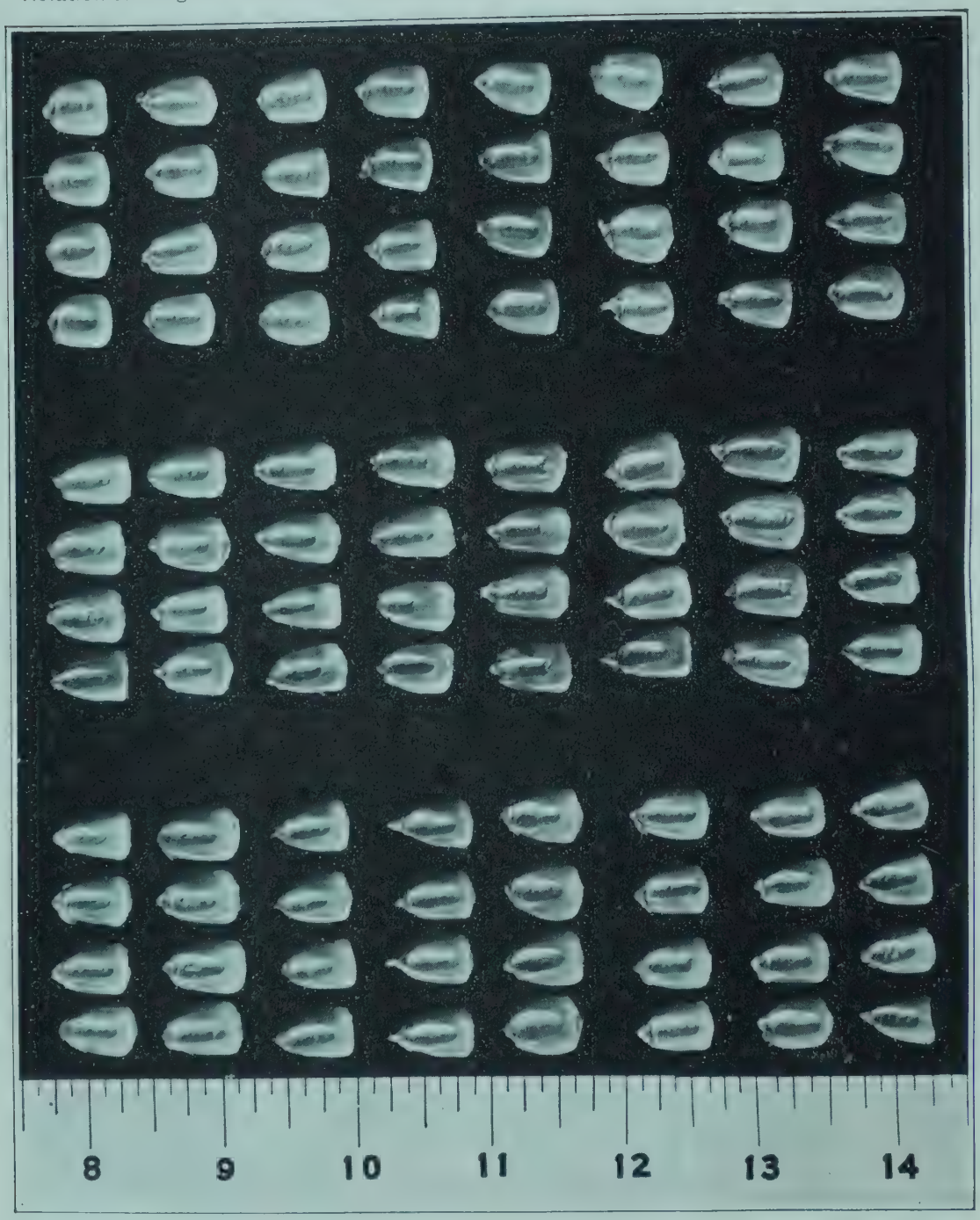
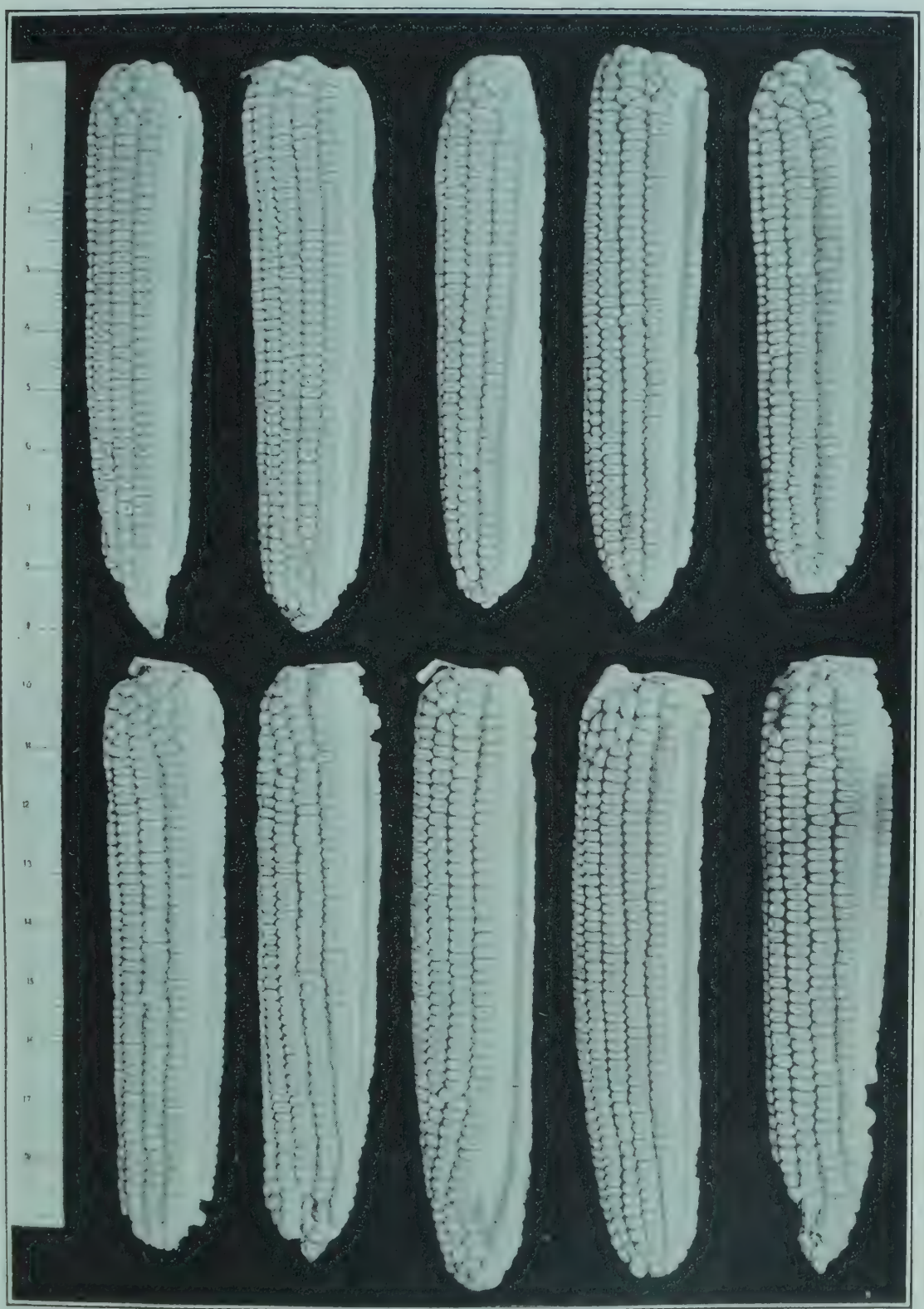

8
9
10
11
12
13
14

PLATE 83

Typical kernels from smooth, rough, and medium ears shown in Plate 80 (left), Plate 82 (center), and Plate 81 (right).

PLATE 84

Typical seed ears of Commercial White corn after four generations in which smooth-dented seed ears were continuously selected.



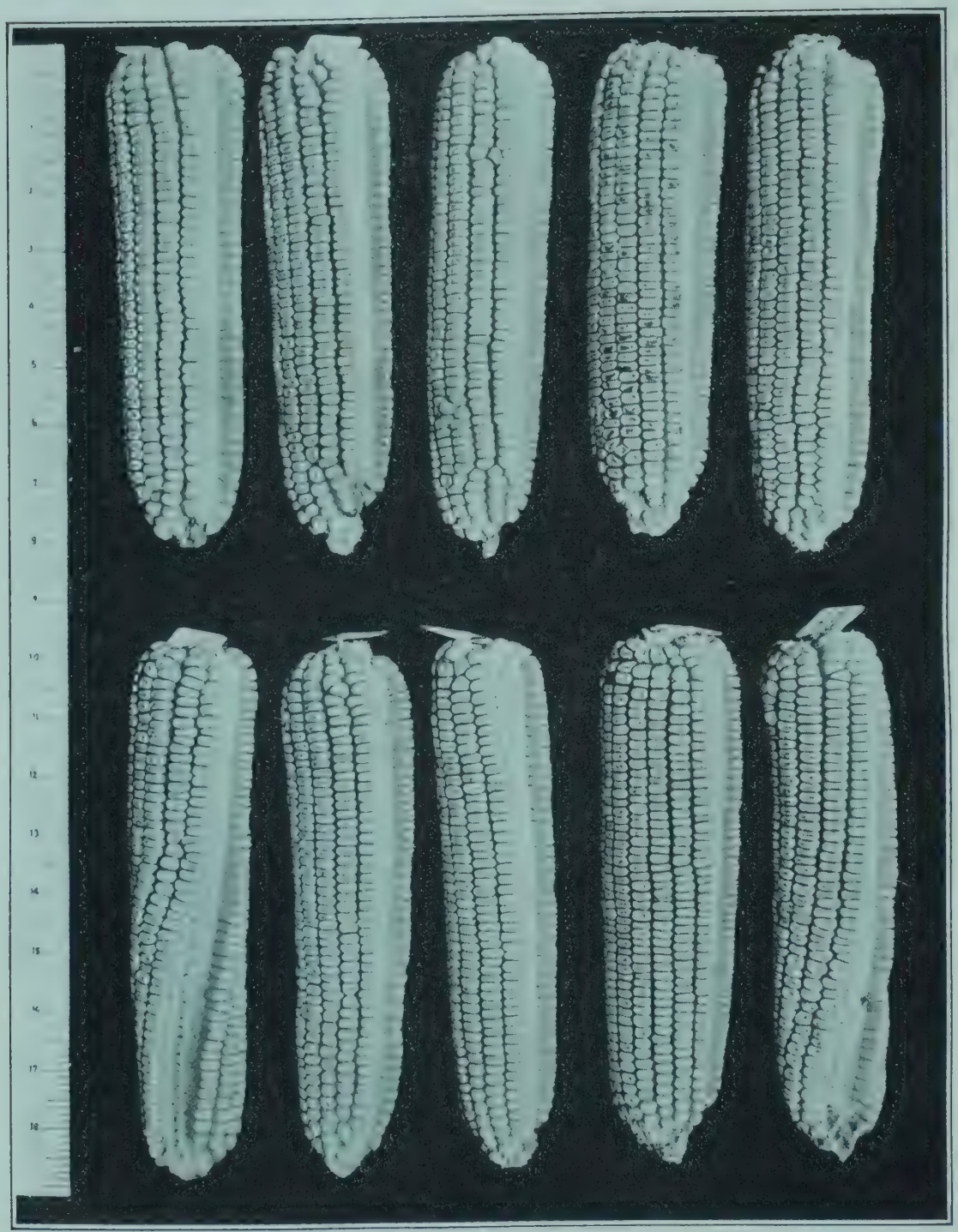


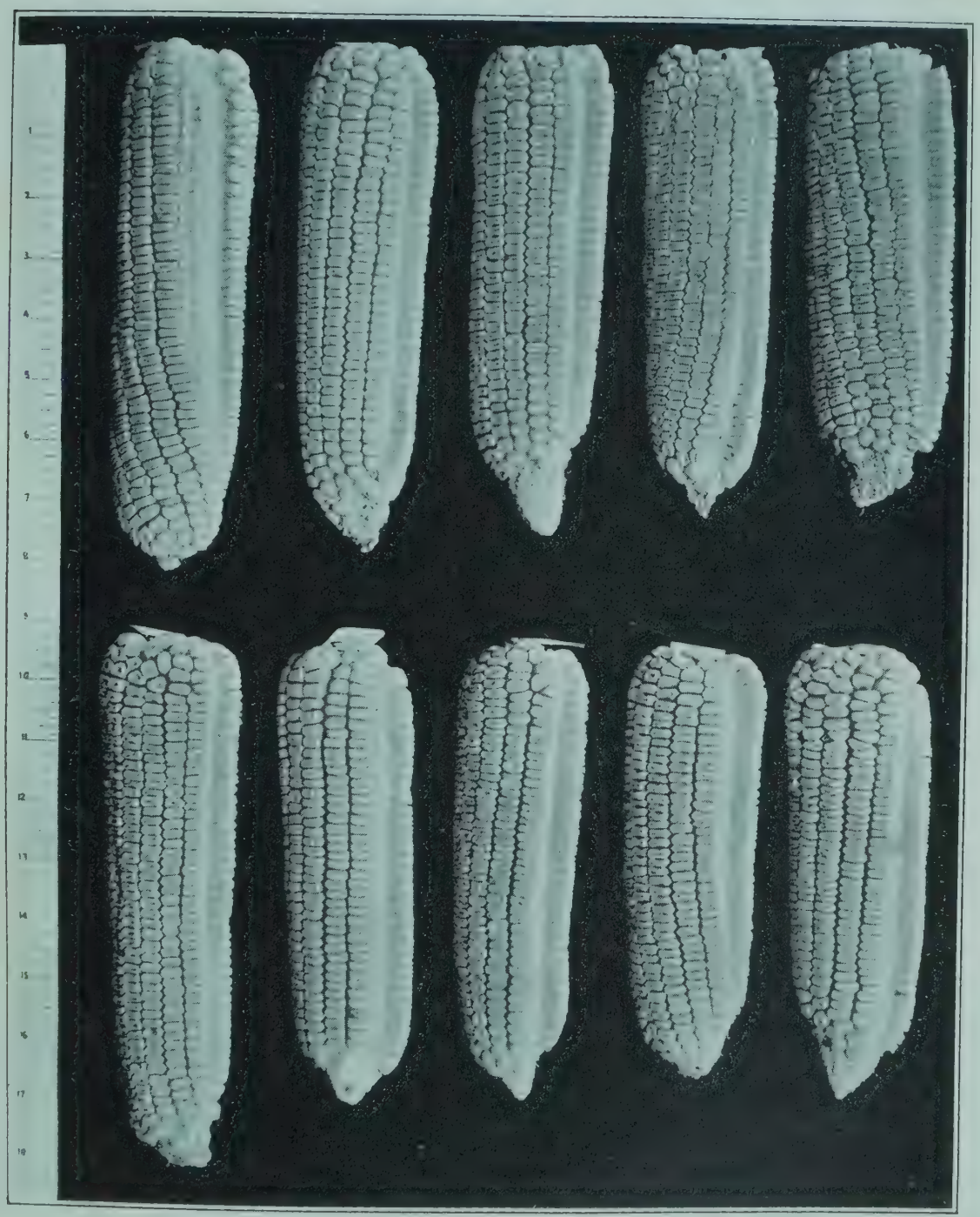
PLATE 85

Typical seed ears of Commercial White corn after four generations in which medium-dented seed ears were continuously selected.

48495°—21——2

PLATE 86

Typical seed ears of Commercial White corn after four generations in which rough-dented seed ears were continuously selected.



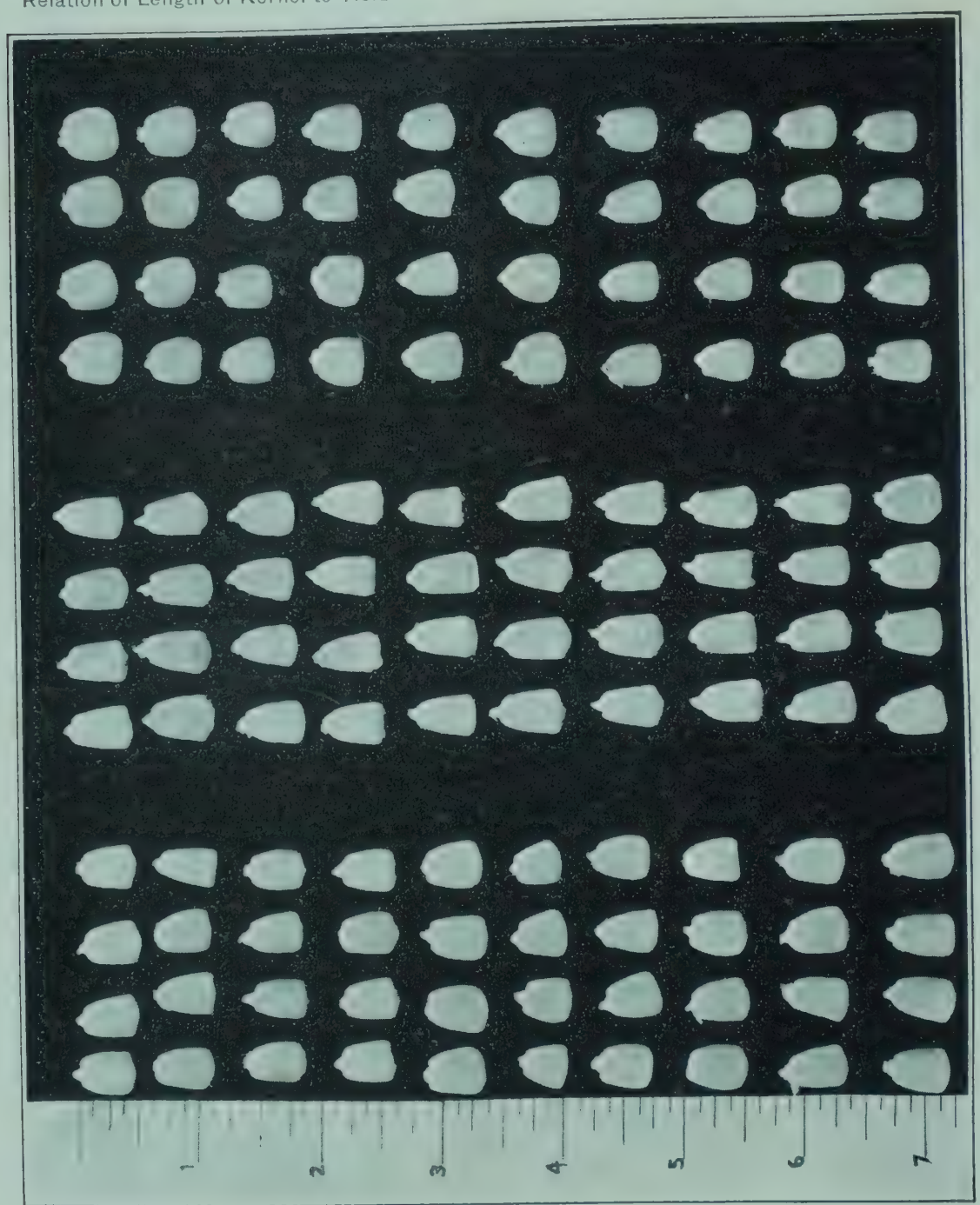

1
2
3
4
5
6
7

PLATE 87

Typical kernels from the smooth-, rough-, and medium-dented seed ears shown in Plate 85 (left), Plate 86 (center), and Plate 85 (right), respectively.

DISTINGUISHING CHARACTERS OF THE LARVAL STAGES OF THE OX-WARBLES HYPODERMA BOVIS AND HYPODERMA LINEATUM, WITH DESCRIPTION OF A NEW LARVAL STAGE

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Since it has been unquestionably shown that the two species of Hypoderma are of great economic importance on account of the injuries caused by the larvæ or grubs in perforated hides, the loss of flesh, and diminished milk production of cattle, it is important that more be known about their morphology in order that they may be distinguished readily in all stages.

The purpose of the present paper is to show the value of the posterior stigmal plates and other characters for differentiating the four larval stages previously known and to describe a new larval stage of *Hypoderma lineatum* De Villiers. In making a careful study of the larvæ it was found that very reliable characters existed for distinguishing larvæ of *H. lineatum* and *H. bovis* De Geer. Separation of these two species in the last two stages was found to be easy by comparison of the form and structure of the posterior stigmal plates. The character of the spiny armature, which was first described by Brauer and which has been entirely relied upon by all investigators up to the present time for the distinguishing of the species, is good only for the last or fifth larval instar; while the characters of the stigmal plates permit, with absolute certainty, the differentiation of the fourth as well as the fifth larval instar.

The stigmal plate is a complicated structure, and it will be considered here only in so far as it is of importance in differentiating the two species in question. J. C. H. De Meijere (*8*)[2] has given an admirable description of the details of structure and functions of the posterior stigmal plates of *H. bovis*.

The plates vary greatly in the different stages of development and to a considerable extent in the same stage. There may even be a marked variation between the two spiracles on the same specimen. Variable as they may appear, however, there are always certain definite associated characters which can be relied upon for each species.

[1] The author wishes to express his thanks and appreciation to Mr. F. C. Bishopp, Entomologist, Investigations of Insects Affecting the Health of Animals, Bureau of Entomology, for his help and suggestions in many ways in carrying out these studies; to Mr. H. B. Bradford, Artist, Bureau of Entomology, who executed the drawings of figures 5 to 23, inclusive; and to Mr. R. W. Wells, Scientific Assistant, Bureau of Entomology, for valuable material collected in various parts of the United States.

[2] Reference is made by number (italic) to "Literature cited," p. 456–457.

The anterior spiracles located above and on either side of the mouth are described in detail by Carpenter and Pollard (*3*). These organs, in so far as they have been studied, do not lend sufficient definite characters to be of any material value in differentiating the two species.

In order to arrive at an interpretation of the structure of the posterior stigmal plates, so far as is necessary in the present paper, it will be best to consider the last or fifth larval instar.

If a transverse section of the stigmal plate is viewed with a medium-power microscope it will be seen that the plate is composed of three layers which may be designated as the external, the middle, and the internal. The internal part, often visible in newly molted specimens viewed from the external surface with a deep focus, is composed essentially of a series of irregular, chitinous, plate-like structures which vary greatly in outline but are fairly constant in number for each species. These radiate from the tracheal chamber. The middle layer is composed of a series of tubes or stems that arise from the internal plate-like structures and terminate at the surface in a disk, the margin of which is most heavily chitinized, giving a ring-like appearance. This is surrounded by supporting tissues. Surface views of the plate often show the separation and even the form of the internal series of structures by light sutures dividing these disks or ring-like structures into sections similar to those in the internal layer, but not so clearly defined.

From surface views of the plates they naturally vary according to the size and number of the disks or ring-like structures, and it is this variation which is so useful in differentiating the larvæ of the fourth instar.

Brauer (*1*, *p. 125*) mentions only three larval stages of *H. bovis*. The first stage he regards as unknown and calls the two large spiny stages appearing in the backs of cattle the second and third. In 1888, Hinrichsen (*6*), a veterinarian in Husum in South Jutland, published an article in which he related his findings of another stage of Hypoderma larvæ in the spinal cavity of cattle. This is the first mention made of what had so far been considered as the first-stage larva or the stage which Brauer regarded as unknown.

In 1890, Cooper Curtice (*4*), of the Bureau of Animal Industry, United States Department of Agriculture, first noted the appearance of a similar stage of a Hypoderma larva which he terms *H. bovis*, appearing in the esophageal walls, under the pleura near the eleventh rib and in the subcutaneous tissue of the backs of cattle. He speaks of this stage as the first-stage larva. This stage was unarmed with spines and unlike the two larger armed stages described by Brauer in the backs of cattle. This was really not the first-stage larva, as C. V. Riley (*9*), of the Bureau of Entomology, in 1891, first described the real first-stage larva of *H. lineatum* which he obtained from the egg just before hatch-

ing. Riley found that this young or first-stage larva was armed heavily with spines and was wholly unlike the much longer, apparently unarmed larva found by Curtice in the esophageal walls or by Hinrichsen in the spinal cavity. Riley states that—

> In the absence of any knowledge of an intermediate form, the larva found in the esophagus may be considered as the second stage

and designates the two stages found by Brauer as the third and fourth stages. In 1914, Gläser (*5*, *10*), of Germany, and Carpenter, Hewitt, and Reddin (*2*), of Ireland, first observed the first-stage larva of *H. bovis* outside of the egg. The three last-named investigators give a complete illustration of the first-stage larva as it appears after emergence from the eggshell.

So far the four larval stages mentioned above have been the only ones known, but during recent studies by the writer for the purpose of determining distinguishing characters of all the larval stages of both species the fact was revealed that there is another stage to be found in the esophagus of the host. This new stage is unlike the small, heavily armed larva that hatches from the egg or the larger, spineless larva that is later found in the esophagus or on the back before molting to the next spiny stage, formerly known as the third stage. Careful examinations were made of a large series of larvæ of all sizes measuring up to 14 mm. in length taken from esophagi and others measuring as high as 16 mm. extracted from the backs of cattle shortly after the hole through the hide had been completed but before the molt to the next larger or spiny stage had taken place. These studies conclusively revealed that the smaller larvæ in the esophagus are not of the same stage as some of the large larvæ measuring from 11 to about 14 mm. in the esophagus and all of those that later appear in the subcutaneous tissues of the back. Since the smaller armed larva found in the esophagus differs from the first stage hatching from the egg it must be considered the second stage, the large, spineless larva in the esophagus and in the back the third stage, and the following two armed stages as the fourth and fifth. There still remains, of course, the possibility of the occurrence of other intermediate stages, especially between the first stage and the first one found in the esophagus. The third or unarmed stage that reaches the back molts from the smaller or armed second stage before it leaves the esophagus, or possibly, in some instances at least, on its journey to the back. Many specimens from gullets as long as 14 mm. are still in the second stage while others similarly located and measuring as low as 11 mm. are in the third stage. The early investigators overlooked the unarmed stage in the back and concluded that there were only two stages encysted under the skin. This oversight was probably due to the fact that at the time the unarmed larva has punctured the hide and is undergoing the molt to the next stage the swelling or the

"bump" of the newly encysted larva is so slight that it remains unnoticed even by a close observer, and later when more growth has taken place and the swelling has enlarged sufficiently to become plainly noticeable the larva is already well advanced in its next stage.

DIFFERENTIATION OF H. LINEATUM AND H. BOVIS IN THE FIFTH INSTAR

In the first, second, fourth, and fifth instars the larvæ of both species bear a heavy, spiny armature in transverse rows, either broken or continuous, across their body segments. Brauer found that by the presence or absence of armature on a certain posterior segment he could definitely identify the larvæ of the fifth instar, heretofore called the fourth stage, by this character alone. In studying hundreds of specimens of both

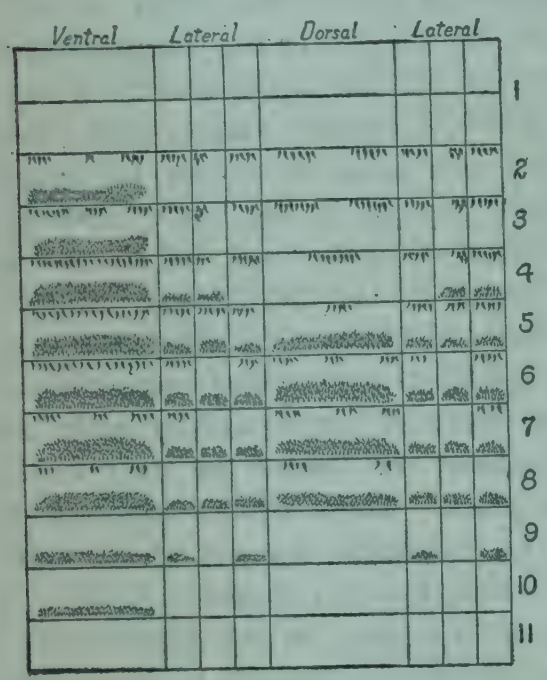

FIG. 1.—*Hypoderma lineatum:* Arrangement of spines on the segments of a fifth-stage larva with a light coat of armature.

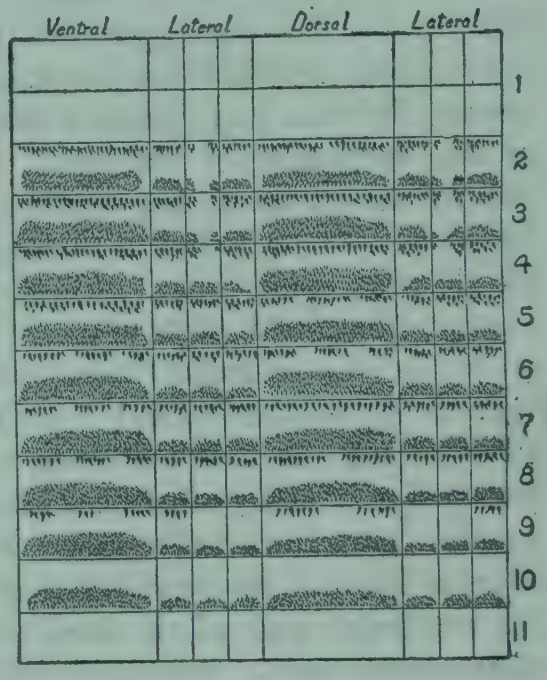

FIG. 2.—*Hypoderma lineatum:* Arrangement of spines on the segments of a fifth-stage larva with a heavy coat of armature.

species the writer found that this character always holds true and is therefore a good one.

The arrangement and variation in the distribution of the spiny armature in fifth-instar larvæ is clearly shown in figures 1 to 4 and Tables I and II. Figures 1 and 2 and figures 3 and 4 represent, respectively, the heavy and light armature of *H. lineatum* and *H. bovis*. The variations presented in Tables I and II are based upon a detailed study of the armature on 106 larvæ of *H. lineatum* and 108 *H. bovis*.

In the tables and diagrams Brauer (1) is followed in considering the first segments as cephalic and marked 1, as these segments are so closely fused and the sutures dividing them are often so indistinct that they appear as one. This method of diagraming the spines is a device adopted by Brauer to indicate the difference in spine distribution in the different

species. The wide spaces of the diagrams represent the ventral surface (on the left) and the dorsal surface (on the right) and the narrow spaces the three rows or lateral divisions. The present diagrams were enlarged by the addition of the three narrow spaces on the right in order to show both lateral surfaces and indicate the variations of the spiny armature so often found on the lateral divisions of the same specimen. The spines on the anterior and posterior borders are represented in size and approximate number as they appeared on the specimens selected for study. On the anterior border the heavier spines curve backward, and on the posterior border the numerous smaller spines curve forward.

By referring to the tables it will be seen that the variations in distribution of spines are more marked on the posterior borders of the seg-

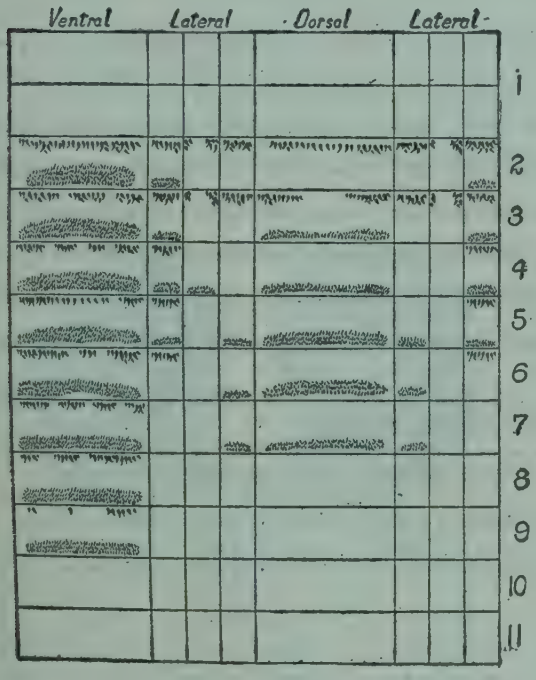

FIG. 3.—*Hypoderma bovis:* Arrangement of spines on the segments of a fifth-stage larva with a light coat of armature.

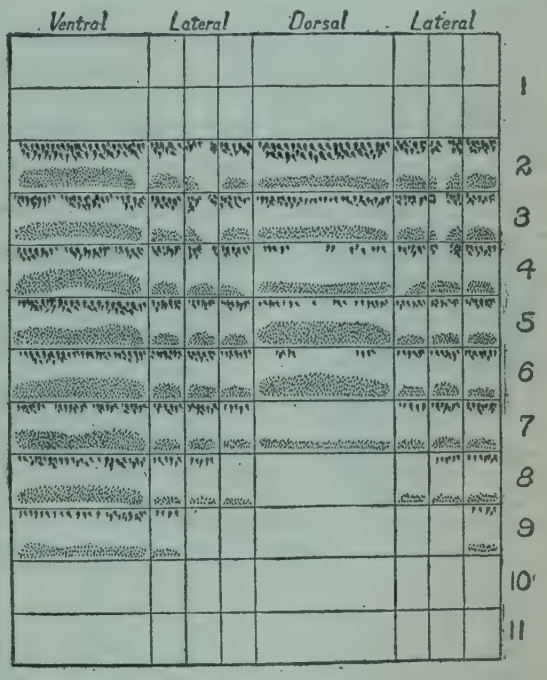

FIG. 4.—*Hypoderma bovis:* Arrangement of spines on the segments of a fifth-stage larva with a heavy coat of armature.

ments except on the ventral surface. On this surface the occurrence of spines is almost constant—that is, all segments from 2 to 10 are armed on their posterior borders in *H. lineatum* and all segments from 2 to 9 are so provided in *H. bovis*. The spines occur on the anterior borders of segments 2 to 9 in both species, only one exception being noted in *H. lineatum*, in which segment 9 was unarmed on this border, and in *H. bovis* one instance in which segment 10 was armed anteriorly, in addition to segments 2 to 9, inclusive. On the lateral divisions of the segments and on the dorsal surface the variation in occurrence of spines is much more marked. It is not uncommon to find certain segments unarmed dorsally while on either side spines are found. The number of larvæ showing such variations is indicated by letters in the figure columns which refer to footnotes.

TABLE I.—*Distribution of spiny armature on the segments of fifth-stage H. lineatum larvæ, based on 106 specimens studied*

Armature on segments No.	Ventral.		Ventro-lateral.		Medio-lateral.		Dorso-lateral.		Dorsal.	
	Anterior.	Posterior.	Anterior.	Posterior.	Anterior.	Posterior.	Anterior.	Posterior.	Anterior.	Posterior.
2 to 10	0	106	0	[a] 19	0	1	0	[a] 32	0	[c] 13
2 to 9	105	0	34	1	0	0	0	[a] 6	[a] 2	[a] 9
2 to 8	1	0	64	0	[a] 20	0	79	0	[f] 88	0
2 to 7	0	0	8	0	29	0	22	0	[d] 16	0
2 to 6	0	0	0	0	48	0	5	0	0	0
2 to 5	0	0	0	0	9	0	0	0	0	0
3 to 9	0	0	0	6	0	[a] 18	0	[c] 11	0	[a] 10
4 to 9	0	0	0	[a] 7	0	[a] 18	0	4	0	4
5 to 9	0	0	0	0	0	2	0	4	0	15
3 to 10	0	0	0	[b] 47	0	[b] 28	0	[c] 41	0	16
4 to 10	0	0	0	[e] 26	0	[d] 34	0	5	0	27
5 to 10	0	0	0	0	0	[a] 3	0	3	0	10
4 to 8	0	0	0	0	0	[a] 2	0	0	0	[a] 2
Total larvæ.	106	106	106	106	106	106	106	106	106	106

[a] One specimen with 1 or more unarmed segments between the first and last armed segments as given in first column.
[b] Two specimens with 1 or more unarmed segments between the first and last armed segments as given in first column.
[c] Three specimens with 1 or more unarmed segments between the first and last armed segments as given in first column.
[d] Four specimens with 1 or more unarmed segments between the first and last armed segments as given in first column.
[e] Ten specimens with 1 or more unarmed segments between the first and last armed segments as given in first column.
[f] Forty-one specimens with 1 or more unarmed segments between the first and last armed segments as given in first column.

TABLE II.—*Distribution of spiny armature on the segments of fifth-stage H. bovis larvæ, based on 108 specimens studied*

Armature on segments No.	Ventral.		Ventro-lateral.		Medio-lateral.		Dorso-lateral.		Dorsal.	
	Anterior.	Posterior.	Anterior.	Posterior.	Anterior.	Posterior.	Anterior.	Posterior.	Anterior.	Posterior.
2 to 10	1	0	0	0	0	0	0	0	0	0
2 to 9	107	108	[b] 29	1	0	0	0	0	0	0
2 to 8	0	0	[d] 65	[a] 89	[a] 2	[d] 43	0	[g] 84	0	82
2 to 7	0	0	11	16	[a] 2	[e] 41	10	[c] 12	0	[a] 18
2 to 6	0	0	3	1	14	1	[a] 18	0	11	0
2 to 5	0	0	0	1	33	[a] 2	[a] 35	0	[f] 27	0
2 to 4	0	0	0	0	55	0	26	0	13	0
2 to 3	0	0	0	0	2	1	18	0	52	0
3 to 7	0	0	0	0	0	[a] 9	0	1	0	2
3 to 8	0	0	0	0	0	[a] 9	1	[c] 6	0	6
4 to 8	0	0	0	0	0	1	0	1	0	0
5 to 7	0	0	0	0	0	0	0	3	0	0
5 to 8	0	0	0	0	0	0	0	1	0	0
2	0	0	0	0	0	0	0	0	5	0
4	0	0	0	0	0	1	0	0	0	0
Total larvæ.	108	108	108	108	108	108	108	108	108	108

[a] One specimen with 1 or more unarmed segments between the first and last armed segments as given in first column.
[b] Two specimens with 1 or more unarmed segments between the first and last armed segments as given in first column.
[c] Three specimens with 1 or more unarmed segments between the first and last armed segments as given in first column.
[d] Six specimens with 1 or more unarmed segments between the first and last armed segments as given in first column.
[e] Seven specimens with 1 or more unarmed segments between the first and last armed segments as given in first column.
[f] Eight specimens with 1 or more unarmed segments between the first and last armed segments as given in first column.
[g] Eighteen specimens with 1 or more unarmed segments between the first and last armed segments as given in first column.

While the presence or absence of spines on the posterior border of the tenth ventral segment is a good distinguishing character of the fifth-stage larva, it is much simpler to distinguish the two species by the shape and structure of the posterior stigmal plates. The flat or level surface of the plate of *H. lineatum* (fig. 5) compared with the distinctly cup-shaped or funnel-form surface of the plate of *H. bovis* (fig. 6) is so well marked that the two species can be identified with the naked eye at sight. If the hair surrounding a warble hole through the skin of an animal is parted and a slight pressure is applied to the skin so as to bring the anal segment of the warble near the surface of the skin it can be viewed through the hole, and a glance is sufficient for determination of the species without extracting it from the host. The size of the stigmal plate of *H. lineatum* is from 0.837 to 1.07 mm. and that of *H. bovis* from 0.971 to 1.27 mm. at its greatest diameter and does not change in size with either species throughout this instar. The structure of the stigmal plates of *H. lineatum* is so formed that the light-colored space between the inward-curving borders around the tracheal opening of the

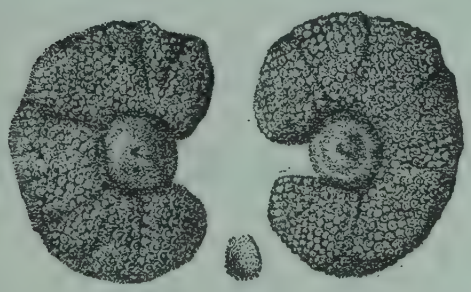

FIG. 5.—*Hypoderma lineatum:* Posterior stigmal plates of fifth-stage larva. Greatly enlarged.

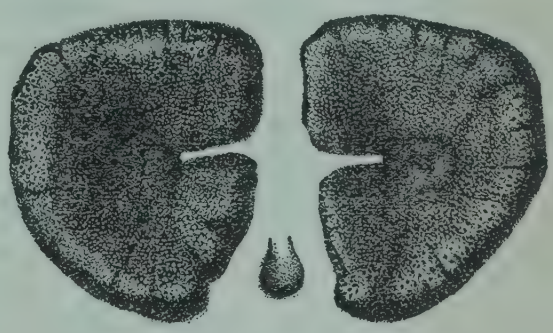

FIG. 6.—*Hypoderma bovis:* Posterior stigmal plates of fifth-stage larva. Greatly enlarged.

respiratory area is usually more than twice as great as the corresponding area in *H. bovis*. This difference is especially noticeable in the younger or lighter-colored specimens. As the larva of the fifth stage grows to maturity and darkens from a light or cream color to black the color of the stigmal plate also changes uniformly, and the age of the specimen can thereby be fairly accurately determined while the grub is still in the host. When the larva has just molted to the fifth instar the color of the plate is light orange throughout. As the larva advances in age the border of the plate turns to a dark brown. A little later the entire plate turns dark brown, and finally just prior to emergence the plate darkens to black. In a series of experiments with various larvicides, conducted at Dallas, Tex., the results of which are to be published later, the color changes of the stigmal plate were very useful in determining the age of the larvæ in the host and the effects of the larvicide on specimens of certain ages. With the dark brown or black larva the finer structure of the stigmal plate is no longer discernible with the naked eye, but the flat surface plate of *H. lineatum* and the cup-shaped

plate of *H. bovis* remain constant throughout the fifth larval instar and also are distinct in the puparia.

DIFFERENTIATION OF THE FOURTH INSTAR OF H. BOVIS AND H. LINEATUM

Available literature on the two species of Hypoderma under consideration gives no description of distinct characters for the identification of the fourth (heretofore known as the third) larval stage. Tabulations of the spiny armature of many fourth-stage larvæ of *H. lineatum* (Table III) and of *H. bovis* (Table IV) revealed that there was no constant character of the armature in either species. The ventral posterior border of segment 10, which is always covered with spines in the fifth larval stage of *H. lineatum* and always naked in *H. bovis*, is nearly always naked in the fourth instar of both species. The armature on the dorsal surface and on the lateral divisions, as given in Tables III and IV, shows that it varies with different specimens of either species as greatly here as it does in the fifth larval stage.

TABLE III.—*Distribution of spiny armature on the segments of fourth-stage H. lineatum larvæ based on 114 specimens studied*

Armature on segments No.	Ventral.		Ventro-lateral.		Medio-lateral.		Dorso-lateral.		Dorsal.	
	Anterior.	Posterior.	Anterior.	Posterior.	Anterior.	Posterior.	Anterior.	Posterior.	Anterior.	Posterior.
2 to 10	1	3	0	0	0	0	0	0	0	0
2 to 9	94	110	0	0	0	0	0	0	0	0
2 to 8	19	1	0	1	0	0	0	[a]1	0	0
2 to 7	0	0	[b]3	1	0	0	0	0	0	0
2 to 6	0	0	[c]37	7	0	0	0	0	0	0
2 to 5	0	0	41	52	0	0	2	0	0	0
2 to 4	0	0	28	8	39	0	55	1	0	0
2 to 3	0	0	5	2	72	0	54	18	2	0
3 to 4	0	0	0	3	0	1	0	1	0	0
3 to 5	0	0	0	22	0	1	0	0	0	0
4 to 5	0	0	0	5	0	1	0	0	0	0
6 to 7	0	0	0	0	0	0	0	0	0	1
2	0	0	0	1	3	3	3	35	69	0
3	0	0	0	4	0	5	0	0	0	0
4	0	0	0	4	0	1	0	0	0	1
5	0	0	0	2	0	0	0	0	0	0
Spineless	0	0	0	0	0	102	0	58	43	112
Total larvæ	114	114	114	114	114	114	114	114	114	114

[a] Segments 4 to 6 unarmed.
[b] One specimen with segment five unarmed.
[c] Three specimens with segment five unarmed.

In studying the characters of the stigmal plate of fourth-stage larvæ collected at Dallas, Tex., and elsewhere in the southern States where only *H. lineatum* exists, and comparing them with specimens collected in the northeastern States where both species are found, it was soon

observed that there was a vast difference in these organs of part of the eastern specimens, and that the others out of the same lots were identical with *H. lineatum* collected locally. Several thousand specimens of fourth- and fifth-stage larvæ collected in all parts of the United States during the past four or five years were all carefully examined, and the fourth-stage larvæ that showed stigmal plates differing from *H. lineatum* were recorded. When these records were then compared with those of the fifth-stage larvæ it was found that all fourth-stage specimens designated as *H. bovis* were from localities where *H. bovis* is present and out of lots that usually contained fifth-stage *H. bovis* larvæ.

TABLE IV. *Distribution of spiny armature on the segments of fourth-stage H. bovis larvæ based on 29 specimens studied*

Spines on segments No.	Ventral.		Ventro-lateral.		Medio-lateral.		Dorso-lateral.		Dorsal.	
	Anterior.	Posterior.	Anterior.	Posterior.	Anterior.	Posterior.	Anterior.	Posterior.	Anterior.	Posterior.
2 to 9	29	29	0	0	0	0	0	0	0	0
2 to 8	0	0	0	0	0	0	0	0	0	0
2 to 7	0	0	0	0	0	0	0	0	0	0
2 to 6	0	0	16	9	0	0	0	0	0	0
2 to 5	0	0	13	19	0	0	0	0	0	0
2 to 4	0	0	0	1	12	[a]1	12	0	0	0
2 to 3	0	0	0	0	17	4	17	5	8	0
2	0	0	0	0	0	15	0	24	18	1
3	0	0	0	0	0	1	0	0	0	0
Spineless	0	0	0	0	0	8	0	3	3	28
Total larvæ.	29	29	29	29	29	29	29	29	29	29

[a] Segment three unarmed.

The characters of the stigmal plates in the fourth-stage larvæ can not be seen or the species distinguished with the naked eye as they can be in the fifth stage, but with the aid of a low or medium power microscope they are distinct. If the specimen is placed under the microscope in a vertical position with the posterior stigmal plates upward and so arranged that they can be viewed at right angles to the surface, the structure is visible. *H. lineatum* (fig. 7) presents a group composed of about 20 ring-like or disk-shaped structures constituting the respiratory areas. These disks, as they may be designated, appear in a group forming a circle or almost any other shape. The color of the disks, which of course is the color of the entire plate, is yellowish or yellowish brown. Most of the individual disks are loosely connected by their borders or are often separated either singly or in small groups,

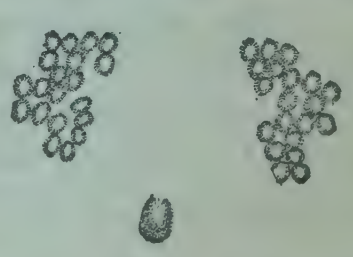

FIG. 7.—*Hypoderma lineatum*: Posterior stigmal plates of fourth-stage larva. Greatly enlarged.

but they are always individually clear and distinct in outline. The color of the stigmal plates remains almost constant during this instar.

While the number of disks is usually close to 20 in each plate it runs as high as 28 or even higher in exceptional cases and as low as 14 in rare instances. The yellowish color and the more distinct separation of the disks readily facilitate counting these structures. The surface of the plates is usually flat in perfect specimens and measures from 0.201 to 0.301 mm. at its greatest diameter.

The characters of the stigmal plate of the fourth-stage larva of *H. bovis* (fig. 8) differ from those of *H. lineatum* (fig. 7) in that the whole plate presents a somewhat larger area which is black and usually contains about 30 disks. The borders of the disks are wider and are all or nearly all heavily fused together so as to present a solid black mass. The number of disks in each plate is also variable, dropping as low as 26 or going as high as 38 in different specimens. The surface of the plate of a perfect specimen is usually convex and projecting slightly above the surrounding tissue. The size of the plate varies from 0.201 to 0.368 mm. at its greatest diameter. With uncleared plates the size of the disks appears to be smaller in *H. bovis* than in *H. lineatum* and they are more indistinct, but after clearing they measure practically the same. The color of the plate remains constant throughout this stage.

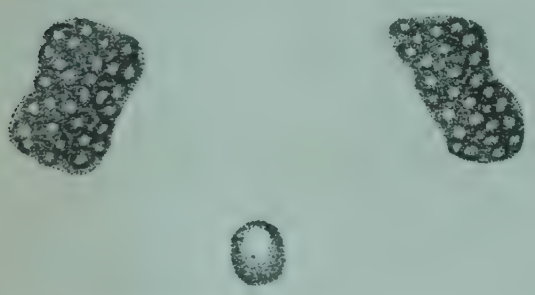

FIG. 8.—*Hypoderma bovis:* Posterior stigmal plates of fourth-stage larva. Greatly enlarged.

Discoloration or dryness of specimens sometimes renders determination rather difficult, but if the surface of the stigmal plate in such instances is kept covered with a film of water or alcohol the characters are usually brought out plainly. Occasionally specimens of *H. lineatum* of the fourth instar are found that show stigmal plates of an almost dark brown color which may at first appear rather confusing, but even in such instances the clear and unfused outline of the individual disks if compared with the solidly fused mass of disks of *H. bovis* remains constant, and it is very easy to determine the species by this character alone.

DIFFERENTIATION OF H. BOVIS AND H. LINEATUM IN THE THIRD INSTAR

The third-stage larva, or the first stage found in the back and heretofore known as the second stage, presents no distinguishing characters in either the structure or the shape of the posterior spiracles or in the scanty, minute armature just below the mouthparts and on the end of the anal segment. The spiny armature is entirely lacking on all the middle segments, and that which appears around the posterior spiracles and

below the mouth is so variable in different specimens that it can not safely be relied upon for separating the species in this stage. If the specimen in question, however, is cleared and mounted and the cephalopharyngeal skeleton and mouth hooks, which can rarely be seen in uncleared specimens, are examined under a medium high power of the microscope, the difference in the structure of the mouth hooks can readily be seen and serves to distinguish the species.

In *H. bovis* (fig. 9–12) the upper or foremost ends of the somewhat shortened semicircular or crescent-shaped mouth hooks (fig. 11, B) are distinctly forked near the tip, and the lower or rear ends of the hooks (fig. 11, C) are blunt. In the illustrations (fig. 11, 15, 22) the mouth hooks are flattened out almost horizontally instead of being in a nearly vertical plane as they are in the normal larva. The articulation of the mouth hooks with the cephalopharyngeal skeleton is in the case of *H. bovis* on the end of a slightly projecting knob of the skeleton turning almost at right angles to the axis, while in *H. lineatum* it occurs on the anterior tip of the skeleton. Between the mouth hooks is a sharp spine (fig. 11; 15; 22, A) directed forward and slightly longer than the foremost tips of the forked mouth hooks. This spine is evidently used to assist in piercing or boring through the tissue while the larva wanders toward the back of the host. After the larva reaches the back and molts to the fourth stage the skeleton with the mouth hooks and spine is cast off.

FIG. 9.—*Hypoderma bovis:* Lateral view of third-stage larva. × 4.

The structure of the mouth hooks of the third-stage larva of *H. lineatum* (fig. 13–16) is more regularly crescent-shaped, is slightly more extended from end to end (fig. 15, B, D), but is otherwise similar in general outline to *H. bovis* with the exception of being distinctly pointed instead of forked like *H. bovis* at the anterior end (fig. 15, B) and having a strong pointed and backward-curving tooth (fig. 15, C) projecting outward about one-third of the length of the entire hook from the anterior tip. The lower or rear end (fig. 15, D) of the mouth hook of *H. lineatum* is also slightly pointed, not as much so as the forward end but considerably more than the rear or backward end of that of *H. bovis*. Out of a considerable number of larvæ of this stage extracted from the back after the hide of the host had been punctured only a few specimens were found that showed a few very minute spines, barely visible under a high power of the microscope, along the anterior borders of the first few segments. If a larva of this stage is placed under the microscope in a vertical

FIG. 10.—*Hypoderma bovis:* Ventral view of third-stage larva. × 4.

position with the anterior end upward and so arranged that the anterior spiracles appear in the center of the field and the mouthparts near the edge, one can see a single, small, but distinct, horn-like appendage at a distance about equal to that of the mouthparts and in a direction from the spiracles opposite from the mouthparts. There are no spines around the base of this appendage, which is smaller and more indistinct in this

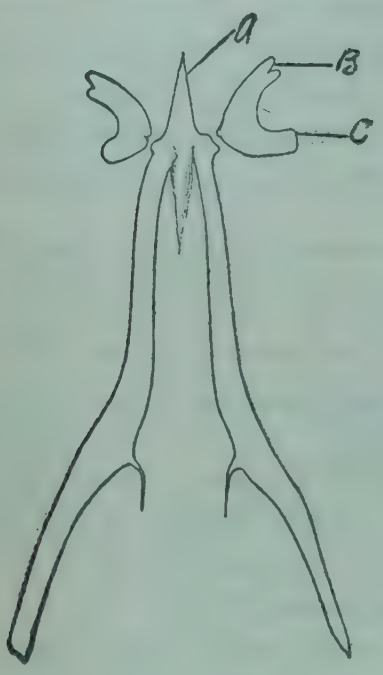

FIG. 11.—*Hypoderma bovis:* Cephalopharyngeal skeleton and mouth hooks of third-stage larva. *A*, spine; *B*, anterior end, and *C*, posterior end, of mouth hooks. × 125.

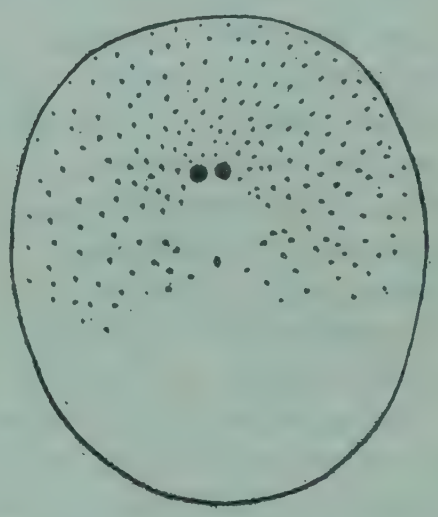

FIG. 12.—*Hypoderma bovis:* Posterior spiracles and spiny armature on posterior half of caudal segment of third-stage larva. Greatly enlarged.

larval stage than in the two younger stages and is entirely absent or invisible in the two larger stages.

DESCRIPTION OF THE SECOND INSTAR OF H. LINEATUM

The second-stage larva has not been recognized heretofore as an instar distinct from that later found in the esophagus and in the back, hence it will be rather fully described. The smaller larvæ found in the gullet are in this stage. Specimens ranging from slightly less than 3 mm. in length and upward have been collected from esophagi of slaughtered cattle at Fort Worth (Tex.) packing houses as early as April 1. During May, 1920, a considerable number of larvæ, all of this stage, measuring from 3 to 6 mm., were taken by E. E. Wehr from esophagi of locally raised cattle slaughtered at a Dallas packing house. These examinations were continued by the writer at weekly intervals. All the larvæ (nearly 400) examined up to August 15, 1920, were found to be still in the second stage. These ranged up to 9 mm. in length. In the general collection made by the writer many other specimens ranging up to as high as 14 mm. in length also prove to be of this stage. All larvæ in this second instar

were taken from the esophagi and were collected in various localities, mostly in the southern States. An effort was made to obtain this stage of both species, but so far the writer's collections of small larvæ in regions where *H. bovis* is found apparently were made too early, and only specimens of *H. lineatum* were obtained. Although a similar stage of *H. bovis* has not been observed so far, it seems reasonably safe to assume that it is also present, since the general characters of the four known larval stages agree in all respects with those of the first, third, fourth, and fifth stages of *H. lineatum*. The comparisons of the larvæ of each stage for the two species under consideration have been based on the assumption that there are five stages of *H. bovis*.

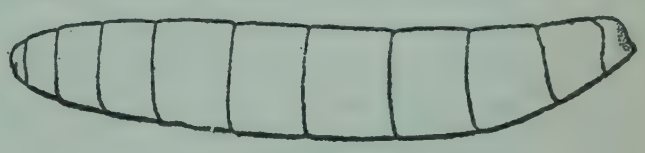

FIG. 13.—*Hypoderma lineatum:* Lateral view of third-stage larva. × 4.

The distinguishing character of this stage lies in the armature which is present on every segment of the entire larva (fig. 17–19). In form, this stage is very similar to the third-stage larva at the time it reaches the back, but of course it is much smaller and not so distinctly segmented. When the armature alone is considered one is led to believe at first sight that it is the first-stage larva which hatches from the egg. After closer examination and comparison with the armature of the first stage, however, it can easily be observed that this is an entirely different coat of armature. The spines not only differ by being smaller in size but also, on the terminal segment at least, differ vastly in structure. While the armature on the middle segments is not as plain as in the first, fourth, and fifth stages, it is nevertheless easily seen with a low power of the microscope and appears in transverse rows very regularly placed on at least the anterior part of each segment. On the second to fourth segments, inclusive, the spines in the anterior row are rather densely placed and are the largest in size. Following the first row are several more irregularly placed rows, in which the spines decrease in size and abundance toward the posterior border of the segment. On the fifth, sixth, and seventh segments the anterior row is also quite regular, but the spines are smaller and farther apart than in the same rows of the preceding segments. The spines behind the anterior row on these middle segments also decrease in number and size in the same proportion as on the anterior segments. On the eighth and ninth segments there is a further decrease in size and number of spines, and the posterior half of the segments is almost naked, except laterally, where small spines extend farther back. The anterior part of the tenth segment is covered ventrally with a band of spines

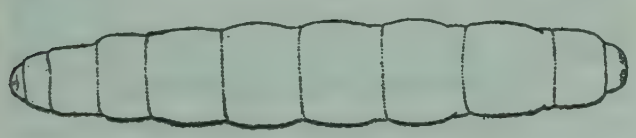

FIG. 14.—*Hypoderma lineatum:* Ventral view of third-stage larva. × 4.

consisting of at least five rows, in all of which the spines are slightly larger and more uniform in size throughout than on the preceding segments. Laterally this band of spines narrows down to the first two or three rows which extend on around over the dorsal side. The eleventh or caudal segment is armed with spines of three distinct types. Along the anterior border are a few small spines similar in structure to those along the anterior border of all the preceding segments. Closely following these, except for a narrow space ventrally, are numerous stout spines consisting of a large, circular, blackish, slightly elevated base, in the interior of which arises a short, stout spine less in length than the diameter

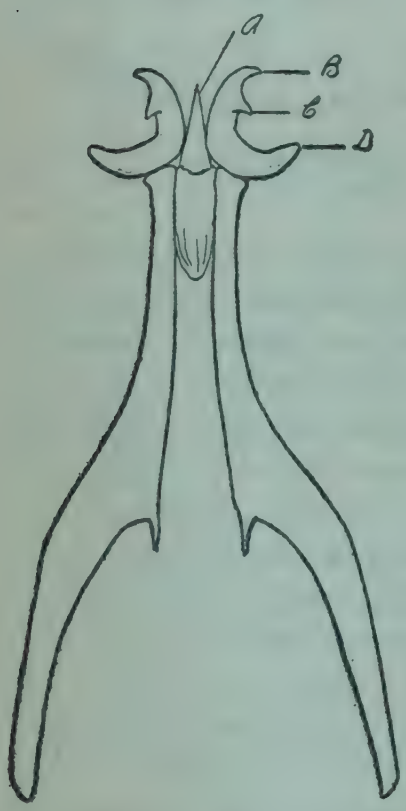

FIG. 15.—*Hypoderma lineatum:* Cephalopharyngeal skeleton and mouth hooks of third-stage larva. *A* spine; *B*, anterior end, and *D*, posterior end, of mouth hooks; *C*, tooth. Greatly enlarged.

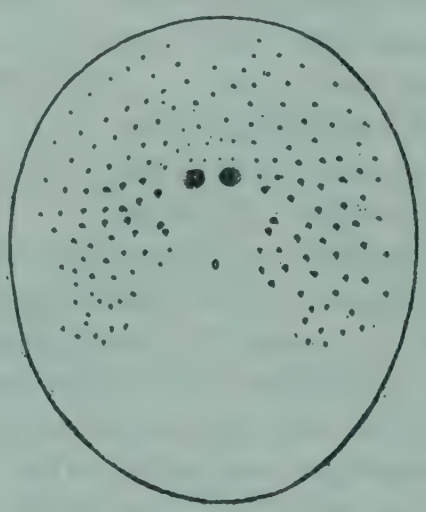

FIG. 16.—*Hypoderma lineatum:* Posterior spiracles and spiny armature on posterior half of caudal segment of third-stage larva. Greatly enlarged.

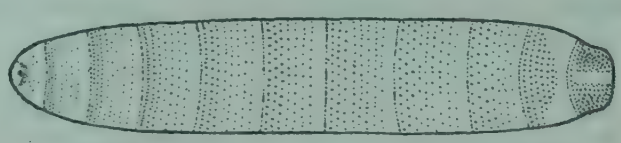

FIG. 17.—*Hypoderma lineatum:* Ventral view of second-stage larva. × 13.

of the base. Surrounded by these spines are the posterior spiracles, which are represented by circular spots or disks with two or three very flattened and triangular-shaped spines on the border of each. These flattened spines are hardly visible in the third-stage larva even under high magnification and disappear entirely with the fourth and fifth stages. The fused anterior terminal segments bearing the anterior spiracles, centrally located from an anterior view, and with the mouth hooks ventrally and the small horn-like appendage dorsally located, are thickly covered with a band of spines below the mouthparts, extending on around and beyond the spiracles laterally in a decreasing number toward the dorsum, where

this band broadens out again and envelops the horn-like appendage. The base of this appendage is surrounded by a dense group of small spines. The posterior border of these segments is covered with small spines, decreasing in number and size toward the border.

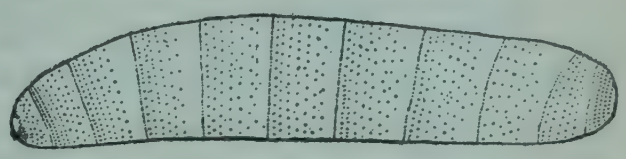

FIG. 18.—*Hypoderma lineatum:* Lateral view of second-stage larva. × 13.

Aside from the greater size of the third-stage larvæ after reaching the back, the second stage may be easily distinguished by the distribution of armature. In the third stage the spines on the cephalic segment are fewer and are clustered beneath the mouthparts and not scattered as in the second instar. In the third stage the armature is absent from all the following segments except the caudal. The few spines that are left below the mouthparts of the third stage and the flattened spines on the border of the posterior spiracles are actually smaller and more indistinct than they are in the second stage, while the numerous spines with enlarged bases surrounding the posterior spiracles of the third stage are considerably larger than those in the second stage.

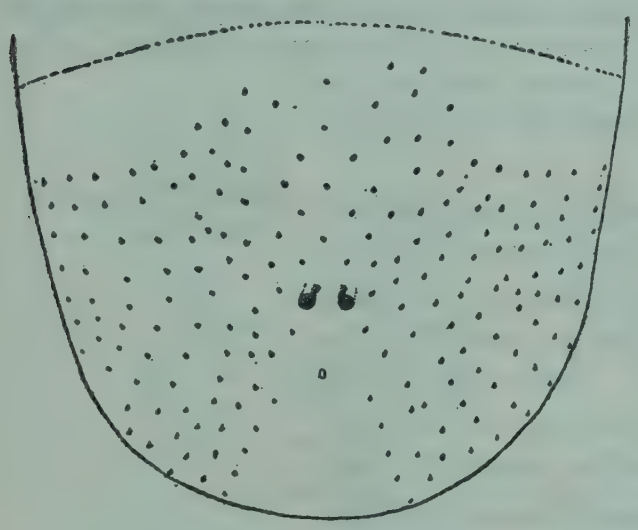

FIG. 19.—*Hypoderma lineatum:* Posterior spiracles and spiny armature on caudal segment of second-stage larva. × 100.

Riley (*9*), in his description of the second-stage larva, states that the larvæ in the esophagus measure from 11 to 14 mm. and are spineless except for a cluster above the mouthparts and the posterior half of the caudal segment. This description agrees in practically all respects with the third-stage larva of both species and is undoubtedly that of a third-stage larva taken from the esophagus. It is certain that Riley would not have overlooked the spiny armature of the larvæ of the earlier stage if any of them had been studied. This may be further substantiated by the fact that the writer has obtained as many as 20 specimens, ranging from 11.7 to 14.6 mm. in length in one lot taken from the esophagus, that were all in the third stage.

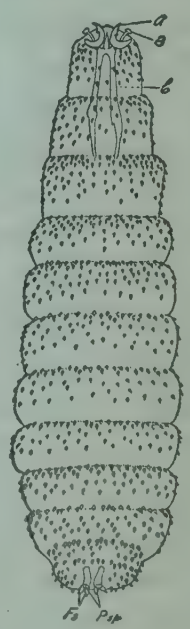

FIG. 20.—*Hypoderma lineatum:* Ventral view of first-stage larva. *A*, Mouth hook; *B*, anterior spiracles; *C*, cephalopharyngeal skeleton; *Fs*, flattened spines on border of posterior spiracles; *P sp.*, posterior spiracles. × 121.

Koch (7), a veterinarian of Denmark, briefly mentions the fact that he observed spines on the very small larvæ which he found in the esophagus and states that this is the first-stage larva. He further claims that the first-stage larva molts for the first time after it has reached the back and has punctured the hide. His reasons for this statement are that—

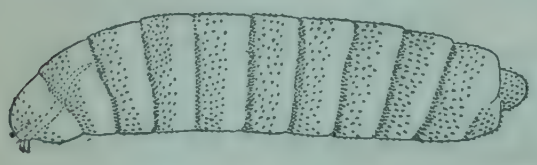

FIG. 21.—*Hypoderma lineatum:* Lateral view of first-stage larva. Caudal segment halfway telescoped. Approximately × 116.

While the larva is in the early part of this stage (first stage) it is clear as glass and transparent, but later it becomes yellowish white, and while the armature of spines is easily observable in the young larva they are less noticeable in the older larva, partly because these are more opaque and partly because the dark color of the spines gradually is lost; and finally it may be stated as a third reason that the spines are not only comparatively but actually smaller in the older larva. It seems as if they become somewhat worn during the migration through the body of the cattle, but it is certain that they remain present and in the same arrangement on the body of the larva throughout this entire period.

FIG. 22.—*Hypoderma lineatum:* Cephalopharyngeal skeleton with spine and mouth hooks of first-stage larva. *A*, spine; *B*, anterior, and *D*, posterior, end of mouth hooks; *C*, tocth. Greatly enlarged.

Attention has already been called to the spiny armature of the second stage or small larva found in the esophagus as the principal difference between this stage and the larger or third-stage larva found in the esophagus and in the back. This alone should be sufficient to convince even the most severe critic that these are larvæ of two distinct stages. However, it may be stated further that if the spiny armature became "worn," as Koch stated, it would nevertheless leave at least a scar or mark in the skin where these spines had been located; but there are none to be found, not even under very high magnification, and it seems impossible that the spines could have been cast off without also casting off the skin. It is true that there is quite a difference in the amount and the arrangement of the spiny armature found on different specimens of the smaller larvæ taken from the esophagus, but this difference is not more marked or unusual than that which is encountered in different specimens of either the fourth or fifth stages, and as a whole the change in character and arrangement of the armature from the fourth to the fifth stage is not as marked as the change found between the second-stage larva in the esophagus and the almost spineless third-stage larva taken from the esophagus or from the back.

DISTINGUISHING CHARACTERS IN FIRST-INSTAR LARVÆ OF H. BOVIS AND H. LINEATUM

Riley (*9*) described the first-stage larva of *H. lineatum* (fig. 20–23) which he obtained from the egg just before hatching and which does not differ in any way from the larva normally hatched from the egg. Careful examinations of some of the hundreds of larvæ of this species hatched from eggs in an electric incubator at the Dallas laboratory agree well with Riley's description; Riley, however, does not mention the anterior horn-like appendage, which is large and very distinct in this stage. The armature on all the segments except the posterior part of the first stage is very dense and considerably larger than on the second stage. On the caudal segment of the first stage the spines with an enlarged circular base are not present, although this segment is well covered with spines similar to but larger than those on the preceding body segments. The flattened spines (fig. 20; 23, Fs) on the border of the posterior spiracles are more than twice as large as the surrounding spines. The dense large spines on the body segments and large flattened spines on the margins of the posterior spiracles of the first-stage larva are in striking contrast with the smaller and much thinner armature on the body segments and small flattened spines on the border of the posterior spiracles surrounded by larger spines with the heavy blackish base in the second stage.

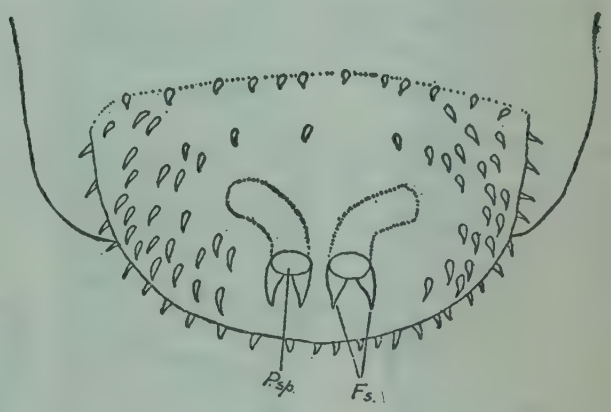

FIG. 23.—*Hypoderma lineatum:* Posterior spiracles and spiny armature on caudal segment of first-stage larva. *P sp.*, posterior spiracles; *Fs.*, flattened spines on border of spiracles. × 500.

The first-stage larva of *H. bovis* (fig. 24) was first described by Carpenter, Hewitt, and Reddin (*2*). The spiny armature on the cephalic and all the body segments is very similar to that of *H. lineatum*, but the arrangement of the spines on the caudal segment differs somewhat, and the spines are considerably larger in *H. bovis*. The flattened spines, usually three in number, on the border of the anal spiracles of *H. bovis* appear smaller in comparison with the large surrounding spines and are actually smaller than in *H. lineatum*. The peculiar type of spines with large circular bases as found in the second and third stages of *H. lineatum* and third stage of *H. bovis* is here absent as it is in *H. lineatum* of the same stage. While the armature of the first-stage larva of the two species in question may differ somewhat, it is hardly reliable for the differentiating of the two species. The characters of the mouth hooks, however, which are here plainly visible in the uncleared specimens with the aid of a

medium power of the microscope, are very reliable and serve to differentiate the species readily. The forked anterior end and the blunt rear end of the mouth hook of *H. bovis* are so distinctly different from the sharply pointed anterior end with a well-formed tooth some distance below and the slightly pointed rear end of the hook of *H. lineatum* that the two species can be separated almost at sight in the first instar.

The mouth hooks of the first, second, and third stages do not change in structure with specimens of the same species except that they become slightly heavier, somewhat shorter, and stouter in the second and third

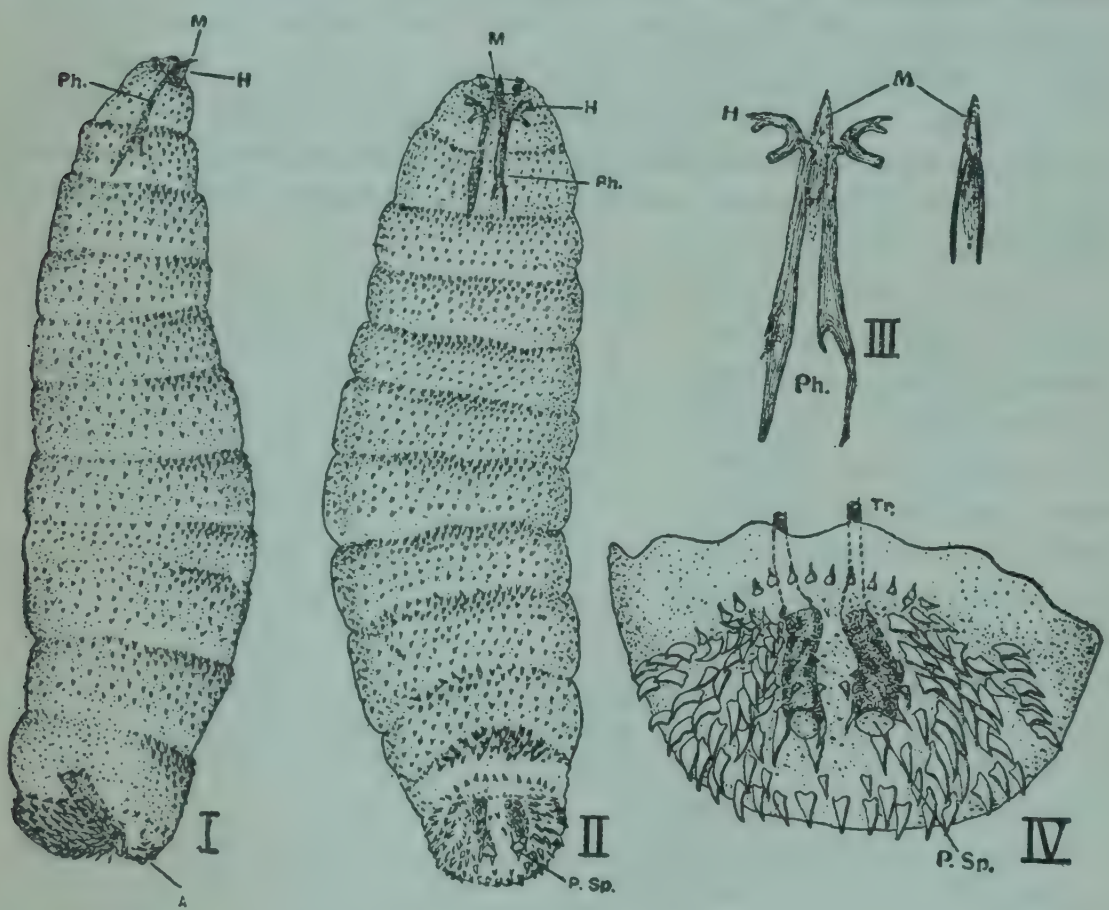

FIG. 24.—*Hypoderma bovis:* First-stage larva. I, Lateral view; II, ventral view; III, cephalopharyngeal skeleton with spine and mouth hooks; IV, caudal segment. *Ph*, Skeleton of pharynx; *H*, mouth hooks; *M*, spine; *Tr*, air tubes; *P sp.*, posterior spiracles. (Carpenter and Hewitt.)

stage, but the two almost parallel rods or arms of the cephalopharyngeal skeleton of the first-stage larva spread considerably posteriorly and become heavier in the second and especially in the third stage.

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PATHOGENICITY OF CORTICIUM VAGUM ON THE POTATO AS AFFECTED BY SOIL TEMPERATURE[1]

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INTRODUCTION

In a paper now in press (*12*)[2] the author treated in detail the question of the pathogenicity of *Corticium vagum* B. and C. (*Rhizoctonia solani* Kühn) and its effects upon the potato plant. Experimental data were presented which showed definitely that under certain conditions the fungus in question becomes an aggressive parasite upon this host. Attention was called, however, to a number of puzzling variations exhibited by this pathogen in its power to attack and produce characteristic lesions on the potato stem. It is frequently noted in the field, for example, that whereas in certain cases the "Rhizoctonia" or the sterile stage of *C. vagum* apparently acts as a definite cankering parasite on potato stems, in other cases where the fungus is present in abundance little or no damage results. The mycelium, in fact, may grow and closely envelop the basal portions of the potato stem as a superificial web with no indication of a lesion or canker. The problem is further complicated by the fact that in certain districts canker-free stems and particularly clean tubers, showing no sings of "scurf," are harvested uniformly from sclerotia-covered seed. Such facts, together with the serious lack of experimental evidence supporting the pathogenicity of the fungus on the potato have led many critical observers to question whether "Rhizoctonia" is of primary or of only secondary importance in the production of the stem cankers so generally attributed to it.

Recent observations in Utah and in Wisconsin have led the workers of these two States to a firm belief that the parasitism of *Corticium vagum* is dependent to a large extent upon soil and climatic conditions. The relative importance, however, of the various factors responsible for the puzzling variation in the pathogenic action of the fungus in question is by no means clear. It has become evident, as previously pointed out (*12*), that further progress on the "Rhizoctonia problem" is dependent upon a better understanding of the biology of the fungus and of the various factors that influence or control its parasitic activity.

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[1] The results here reported are based upon greenhouse investigations made during 1917, 1918, and 1919, at the Department of Plant Pathology of the University of Wisconsin. During the seasons of 1918 and 1919, soil temperature studies were made with the potato under natural field conditions. These studies, together with greenhouse experiments on the pea and bean, paralleling those on the potato, will furnish the basis for later reports. The writer wishes to express his indebtedness to Prof. L. R. Jones for helpful suggestions and criticisms during the progress of the work.

[2] Reference is made by number (italic) to "Literature cited," p. 481-482.

A recognition of this fact, together with the opportunity offered at the Department of Plant Pathology of the University of Wisconsin, led the writer to conduct a series of studies upon soil temperature in its various relations as affecting the pathogenicity of *Corticium vagum* upon the potato and other hosts. The purpose of this paper is to report part of the results of these investigations.

One of the most important features of the recent development in phytopathology, as pointed out by Jones (*6*), has been the directing of the attention of workers in this field to the vital relation of the temperature of the soil to a large number of our most serious soil diseases. Our present knowledge on this subject is at best limited and fragmentary, and the degree to which soil temperature becomes a rigid controlling factor in determining pathogenicity is known for but a small number of soil pathogens. These data do not permit of extensive generalization. It is evident that *Corticium vagum*, with its wide range of hosts, offers an especially favorable object for the study of parasitism in relation to the temperature of the soil.

REVIEW OF LITERATURE

Observations on soil temperature in relation to the pathogenicity of *Corticium vagum* on its various hosts are few and conflicting. Rolfs (*13*, *14*) concludes that—

> a high temperature and plenty of moisture are necessary for the rapid development of the fungus—

and correlates the high death rate of potato plants in Colorado with excessive irrigation during periods of hot weather.

Peltier (*11*, *p. 283-285*), in his greenhouse experiments with the carnation and other hosts, obtained a higher degree of infection with the fungus during the months of June and July and September and October than during the cooler months of spring and winter. He supplies other minor data to support this relationship and finally concludes his observations as follows:

> A high temperature, 88° F., together with either too little or too much moisture, determines to a large degree the virulence of the strains.

It is evident that this writer has reference here to air temperatures and, therefore, adds but little to our knowledge of the exact soil temperatures conducive to the pathogenicity of the various strains of the fungus. In his later statement we are again left in doubt as to whether he has in mind only one or the many hosts with which he worked.

Morse and Shapovolov (*8*) noted that in a certain potato field held under their observation 50 per cent of the plants showed lesions in the middle of July, while on August 4, 91 per cent showed evidence of attack by the fungus. No temperature data were presented, and it is not clear that temperature was an important factor in this relationship, since length of

exposure, soil moisture, and the growth of the new tissues, together with other possible factors, might easily have accounted for this observed difference.

Balls (*1*), on the other hand, presents data from critical field observations which indicate definitely that cool temperatures favor the pathogenic action of the fungus *Rhizoctonia solani* on cotton. The data presented also suggest that the cool weather increases the severity of attack both by retarding the growth of the young cotton seedlings and by favoring definitely the action of the fungus upon the plant tissues. At the higher soil temperatures it appears the plant may escape unharmed even in a heavily infested field. This writer states his conclusions as follows:

> There is no doubt, in view of this year's experience, that given a supply of healthy mycelium in the soil of average texture, the amount of damage done to the cotton crops depends upon the temperature of the soil.

By pure culture work Balls (*2*) was further able to show that "Rhizoctonia" could attack the cotton plant vigorously at 22° C., but he obtained no infection at 33° C.

GENERAL CONSIDERATION OF THE FACTORS INVOLVED

Progress in the analysis of so complex and fluctuating a condition as is found in parasitism can be made best by the common procedure of varying one factor in the environment while holding all others under control. Because of the complex nature and the interrelation of the factors involved, an absolute control of the possible variable is rendered difficult if not impossible. Proper evaluation must be given those variations outside the limits of control. The problem, however, becomes further complicated in that the variables concerned must be controlled as nearly as possible, at a range approximately optimum for the parasitic relation under consideration. Wholly safe conclusions as to the importance of any one factor are possible, therefore, only when the value of several of the most important relations contributing to a parasitic state have become known.

Such factors as are concerned in influencing the parasitic action of *Corticium vagum* on the potato might be considered as both hereditary and environmental in nature. Of the former group, strain differences of the fungus and host susceptibility are of first importance. The controlling features in the environment, however, are more numerous and may be listed for convenience as follows: (1) the oxygen supply of the soil, (2) the soil temperature, (3) the soil moisture, (4) the acid or alkaline reaction of the soil, (5) the organic matter in the soil, (6) the soil texture, and (7) the soil flora and fauna, including the degree of soil infestation of the strains of the parasite under investigation. At present, the true relation and importance of any one of these various factors to the parasitic

state existing between *C. vagum* and its numerous hosts is not known. In the present soil temperature studies the use of the same soil and a single strain of host plant made the problem of controlling the various influencing factors relatively simple. The methods employed are discussed below. In general, an attempt has been made to depart as little as possible from the natural conditions of potato culture.

GENERAL METHODS AND MATERIAL

APPARATUS

The experiments were conducted in especially constructed water jackets now in use for soil temperature studies in the greenhouses of the Department of Plant Pathology of the University of Wisconsin. The essential features of these were described and illustrated by Jones (*6*) in 1917. Johnson and Hartman (*5*) in 1918 gave further details as to their structure and convenience for temperature control. It should be mentioned here that each unit or tank as used in the experiments consists of an insulated water chamber 3½ feet long, 2 feet wide, and 1½ feet deep. Eight cans 7 inches in diameter and 12 inches deep were so arranged in each water jacket as to form two parallel rows of four cans each. The cans were supported in the water to a depth which just permitted of safety from overflow. The ½ inch to 1 inch of the cans thus remaining above the water projected into the hole in the cover in such a way as to provide for ample lateral support. Cold water and steam pipes with outlets were arranged in connection with the tanks so as to permit of the convenient use of either steam or water. Electric heating units were also placed at the bottom of the water jackets that were kept at the higher temperatures. Each water jacket thus equipped became an independent unit, with a possible eight pots of soil all held at the same temperature for the growth of plants. The range of temperatures within the temperature limits of plant growth, therefore, was limited only by the number of units em-employed.

TEMPERATURE CONTROL

The large number of tanks rendered the use of automatic temperature regulation impracticable. It was found, however, that by personal adjustments three times during the 24 hours at intervals of approximately 8 hours each, the temperature could be controlled within a range of variation sufficiently narrow to be adequate for obtaining the required data. At each visit the various water temperatures were recorded and proper adjustment was made. At those temperatures which approached the temperature of the greenhouse, the water was adjusted to the exact degree desired for the soil. At the lower and higher extremes, however, it was found necessary to raise or lower the temperature of the water

sufficiently to counterbalance the amount of variation above or below that which was desired.[1]

At the highest temperature the water was maintained at an extreme range of variation of 3°, or from 28.5° to 31.5° C. At 27° this range of variation was less than 1.5°, while at 24° and at those temperatures approaching that of the greenhouse the total temperature variation rarely exceeded 1°. With a mat of wet sphagnum on the cover of the tanks and with high collars around each can, the variations at the lower temperatures of 9° and 12° were held within a range of less than 2°. Under the system of regulation, the extremes of the variations were of relatively short duration.

Owing to the direct exposure to the surrounding atmosphere the surface of the soil in the cans held at the extreme high and low temperatures varied somewhat below and above that of the water in the respective tanks. These differences could not be detected at a soil depth below 2 inches. Since the potato sets were planted at a depth of 5 inches and since no variation in the vertical distribution of stem lesions could be detected, the average mean temperature of the water in each tank was taken as the temperature of the soil[2] at which the plants were grown. The air temperatures in thee greenhouse at which the plants were grown were maintained in general between 17° and 22° C.

SOIL MOISTURE

Determinations made at the beginning of each experiment showed the moisture content to vary in all experiments between 20.4 per cent and 24.8 per cent of the dry weight of the soils used. This range of soil moisture had been previously determined to permit of uniform infection of potato stems, and no further precaution was taken to regulate more definitely the relation of the moisture content in the soils in the various experiments. The maintenance of a constant water relation throughout any one series, however, became a much more complicated process. The large number of cans used in the experiment and the relatively short period of growth of the plants rendered the use of the Livingston auto-irrigators (7) impracticable. The direct weight method was finally adopted. In this method precautions were taken at the beginning of each experiment to obtain a uniform water content of the soil used in any experiment. In the process of planting, all cans were then filled to exactly the same weight, and during the progress of the experiment the

[1] At the higher temperatures, the variation in the temperature of the water in all cases fell below those desired. The reverse was true for those below the temperature of the greenhouse. The adjustments found necessary were as follows:

Desired temperature—30°, 27°, 24°, 21°, 18°, 15°, 12°, 9° C.

Adjusted temperature—31.5°, 28°, 24.5°, 21°, 18°, 15°, 11.5°, 8° C.

[2] The figures recorded in all tables must be considered as the exact calculated average of the soil temperatures at which the crops were grown and should not be assumed to indicate a degree of accuracy in temperature control as might be implied by the retention of the fractions given in the tables.

water relation was kept constant by frequent weighing and addition of water to restore that lost by evaporation from the soil or through transpiration by the plants. The weighings were usually made every two or three days; at the higher temperatures water was added more frequently as required.

SOURCE OF FUNGUS

A single strain [1] (201) of sterile stage of *Corticium vagum* was used in all the experiments in which artificial methods of soil inoculation were employed. The fungus was increased on bean pods and was introduced into the soil usually after about six weeks' growth on this medium. In three experiments the soil was inoculated by sclerotia upon the surface of the tubers used for seed.

SOIL INOCULATION METHODS

The methods of soil inoculation followed were essentially those described for the work on the pathogenicity of the fungus. They need not be discussed here. In general, where artificial methods were employed the inoculum was placed below the potato sets so as to make sure that none of the material introduced would come in contact with the growing stems. The sets in all cases were planted at a depth of 5 inches. After planting, the cans were allowed to remain under uniform condition for a period of six days, at the end of which time the temperatures were adjusted in the various tanks.

In removing the plants at the conclusion of each experiment the soil in each can was first saturated with water for a period of 24 hours. The roots and stems were then washed free from dirt by a gentle stream of water from the hose.

QUANTITATIVE INDEX

Previous field and greenhouse experiments had shown clearly that owing to the peculiar nature of the attack of *Corticium vagum* (*12*) the percentage of the potato stems showing lesions gave no accurate idea of the damage resulting to the plants. The degree of the intensity of injury to the stems was used, therefore, as an index for determining the different temperature values (Pl. 89–93). In an attempt to express this relationship the diseased stems occurring at each of the various temperatures were divided into three categories: Slightly injured, severely injured, and cut off. All stems showing lesions ranging from the first indications of disease to those sufficiently serious to interfere with the physiological processes of the plant were listed as slightly injured. Stems

[1] This strain is the same employed in the previous experiments on pathogenicity, for the record of which the reader is referred to the earlier paper (*12*). The term "strain" is used here with no special morphological or physiological significance. It indicates, rather, the results of a single isolation from a definite locality.

showing lesions which either from depth of penetration or from extent of surface destroyed appeared definitely to interfere with the activity of the plant were classed as severely injured. In the third group were placed all stems showing definite growing-point destruction and also those which had actually been cut off by the operation of the fungus subsequent to the appearance of the stem through the soil. In order to obtain a quantitative expression of the intensity of injury at each temperature, the percentage of plants occurring in each class was determined and units 1, 2, and 3 were allowed for each percentage in the three classes slightly injured, severely injured, and cut off.

EXPERIMENTAL STUDIES

In all the experimental work with the potato, the application of the results to the interpretation of the operation of the fungus under natural field conditions was kept constantly in mind. In order to eliminate all possible interfering organisms and to establish a firm basis for judgment, experiments were first conducted with pure cultures of the fungus in sterile soil. The results obtained under these extremely artificial conditions were finally supplemented by those obtained with the temperature equally well controlled, but with other relationships of the host and the fungus approaching as nearly as possible those found under natural conditions. As a final control the temperature work was carried into the field, where plantings and inoculations were made at definite intervals and the temperatures recorded throughout the growing season. In addition, valuable temperature data were obtained during 1918 and 1919 from the disease-control experiments conducted on a rather extensive scale in important potato-growing districts of northern Wisconsin. These latter field studies as stated will furnish the basis for a later report.

EXPERIMENT 1.—In the first experiment four tanks with eight cans each were used. The water in the individual tanks was held at approximately 16°, 19°, 23°, and 29° C.

The soil used was sterilized with steam for 7 hours at two different periods 24 hours apart and at a temperature of approximately 97° C. Two half tubers of the Irish Cobbler variety, treated previous to cutting, were then planted with the inoculum in each of six of the eight cans in each tank. Two cans in each tank were left for the growth of control plants in uninoculated soil.

Thirty-eight days after planting the plants were removed and examined. The total intensity of stem injury together with the percentage of stems injured is shown in Table I and graphically in figure 1.

The most serious types of lesions occurred unmistakably at the two lower temperatures. Growing-point injury was found only at the lowest temperature of 16° C. The injury on the stems at both 24° and 29° was very slight.

TABLE I.—*Effect of growing Irish Cobbler potatoes at different temperatures in soil inoculated with a pure culture of Corticium vagum*

EXPERIMENT 1

Temperature (° C.).	Number of sets planted in each can.	Number of stems grown in uninoculated soil.	Stems grown in inoculated soil.						
			Total number.	Number slightly injured.	Number severely injured.	Number cut off.	Number uninjured.	Percentage injured.	Intensity of injury (points).
16.....	2	11	33	7	17	2	7	78.7	142.2
19.....	2	9	21	7	5	.....	9	56.6	80.0
23.....	2	12	22	14	.....	.....	8	66.2	66.2
29.....	2	4	16	4	.....	.....	12	25.0	25.0

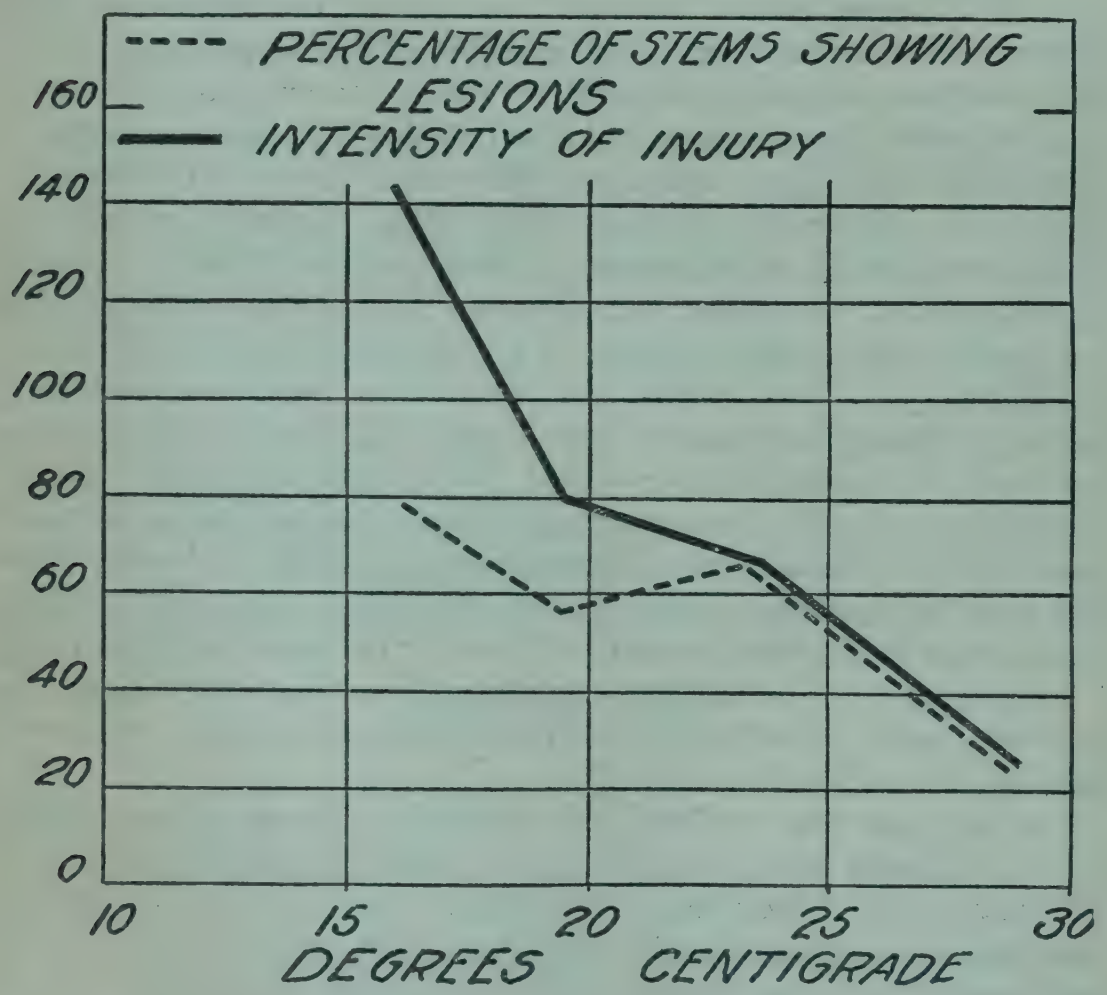

FIG. 1.—Relation of soil temperature to the severity and to the distribution of the injury on potato stem caused by *Corticium vagum* (experiment 1).

All control plants remained entirely free from lesions except those in one can in each of the two tanks held at the two lower temperatures. These plants showed definite brown cankers accompanied by the characteristic mycelium of *Corticium vagum*. The lesions, however, were relatively slight and indicated a possible late infection. The accidental inoculation of the soil in these control pots may have resulted in one of three ways: (1) From imperfect surface sterilization of tubers used for

seed, (2) from the growth of the fungus from the inoculated to the control pots through the moist sphagnum used for insulation, or (3) from the basidiospores from the corticium stage of the fungus which occurred abundantly during a warm period in all the aerial parts of all the inoculated plants. The last explanation appears the most probable.

EXPERIMENT 2.[1]—The results obtained in experiment 1 indicated that the fungus is capable of causing diseases at a temperature much lower than 16° C. It was decided for subsequent experiments to include the possible temperature range at which the potato plant might be grown profitably. This plan resulted in increasing the number of tanks in the series to eight. These were maintained approximately at the following temperatures: 9°, 12°, 15°, 21°, 24°, 27°, and 30°. The 8 tanks containing 8 cans each were placed side by side, making four series of 16 cans, 64 in all. Throughout this and subsequent experiments one series of 2 cans in each tank was used for growing control plants. The other three series were used for growing plants in inoculated soil.

In this experiment two series were run with sterile soil. In a third series unsterilized soil was used. The sterilized and unsterilized soils were represented separately in the two control cans in each tank. Treated southern-grown Irish Cobbler potatoes were used for seed. These had begun to sprout and were in good condition for growth. The results are presented graphically in figure 2.

TABLE II.—*Effect of growing Irish Cobbler potatoes (southern seed) in soil inoculated with Corticium vagum and held at various temperatures*

EXPERIMENT 2

Temperature (° C.).	Number of sets planted.	Number of stems grown in uninoculated soil.	Stems grown in inoculated soil.						
			Total number.	Number slightly injured.	Number severely injured.	Number cut off.	Number uninjured.	Percentage injured.	Intensity of injury (points).
9	1	5	17	.....	5	.....	12	29.7	59.4
11.6	1	4	13	2	.....	.....	11	15.0	15.3
15	1	5	12	2	1	.....	9	24.9	33.2
17.4	1	5	14	1	5	4	4	71.2	153.8
21	1	6	10	1	6	.....	3	70.0	130.0
23.7	1	8	14	.....	3	.....	11	21.4	21.4
26.8	1	5	12	2	1	.....	9	24.9	33.2
30.3	1	6	10	.....	.....	.....	10	.....	.....

At the conclusion of the experiments the plants grown at the temperatures from 15° to 30° C. ranged from 2 to 5 inches high. Those grown in the 12° tank were about 1 inch above the soil line, while but few in the

[1] In an experiment run immediately after experiment 1 and under conditions as outlined in experiment 2 the inoculum, consisting of the fungus on sand-cornmeal mush, was mixed throughout the first 5 inches of soil. The results were so irregular and the root system of the inoculated plants was so unbalanced that the data were discarded. The experiment is not given a number in the series of tests. The control plants of this experiment were used for the measurements given in Table X and were photographed (Pl. 88).

tank held at 9° had succeeded in getting above the surface. The plants were thus removed at a period too early to obtain reliable data as to the effects of the fungus on the shoots above the ground. The results of the observation made 30 days after planting are shown in Table II.

The results were surprising, in that stem lesions occurred throughout the entire range of temperatures from 9° to 27° C. No lesions were found on the stems grown at a soil temperature of 30°. The greatest

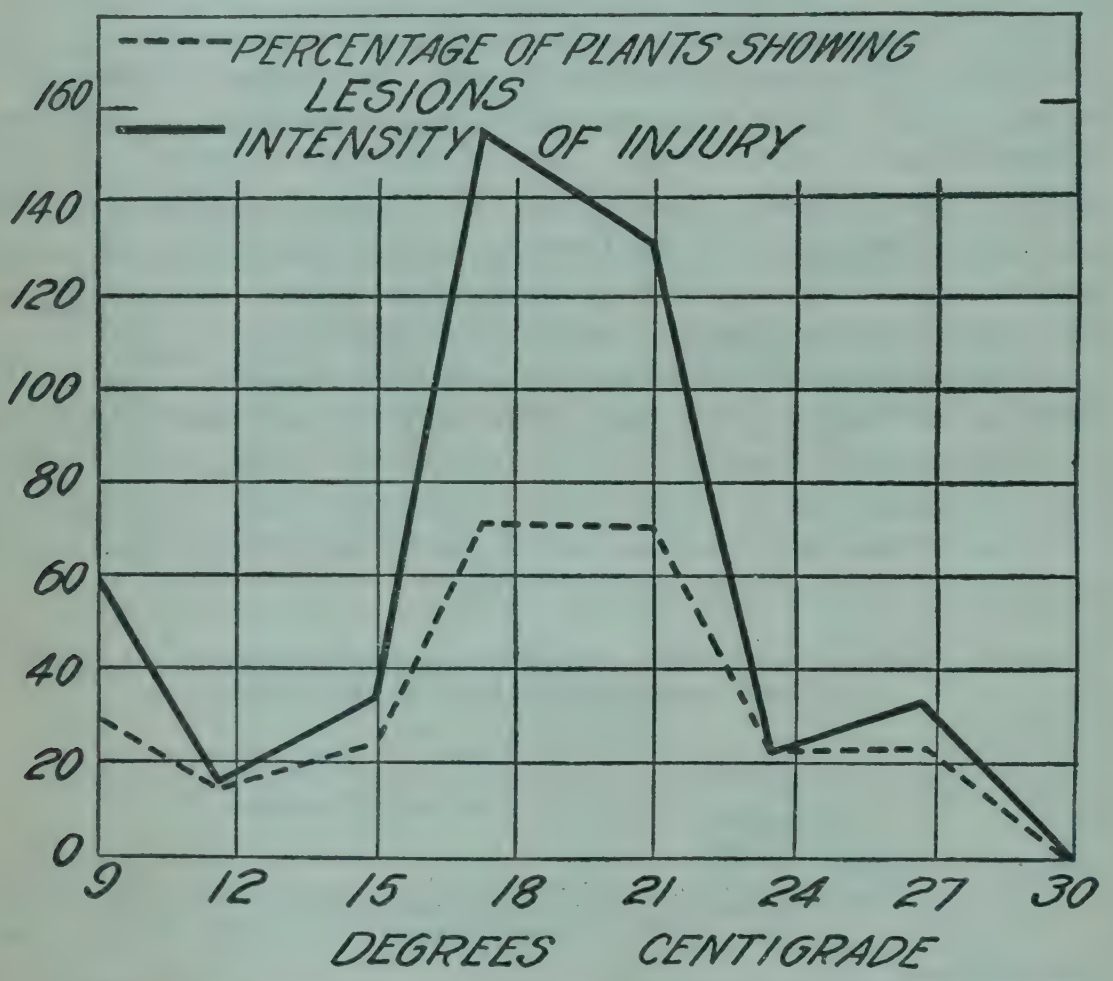

FIG. 2.—Relation of soil temperature to the severity and to the distribution of the injury on the potato stems caused by *Corticium vagum* (experiment 2).

degree of injury occurred unmistakably at 18°. The plants in the soil held at 21° were also severely damaged.

Decidedly greater damage occurred in the unsterilized as compared with the sterilized soil. All plants in the control cans remained free from lesions.

EXPERIMENT 3.—The results thus obtained with pure cultures in sterile soil show definitely the power of the fungus to produce lesions on potato stems through a range of soil temperature from 9° to 27° C. The conditions, however, under which the data were obtained were obviously artificial and can provide but a very general idea as to what might be obtained in nature. With the view of obtaining a more accurate index to the practical problem, natural means of soil infection were used in this experiment.

Unsterilized soil was again used in one series of 16 cans, 2 cans at each temperature. A second series was provided with steam-sterilized soil. As in experiment 2 both types of soil were represented in the controls.

As the Irish Cobbler potatoes used for seed were free from sclerotia, two small Early Ohio tubers well covered with sclerotia and without treatment were placed in the bottom of each can and covered with approximately 1 inch of soil. Three halves of Irish Cobbler tubers, previously treated, were then placed on this surface and covered to the regular depth of 5 inches. All the controls were arranged in exactly the same way except that the potatoes used were treated before planting. Observations made 42 days after planting are recorded in Table III.

The plants, on the whole, exhibited a more severe type of injury in the sclerotia-inoculated soil than was obtained with the pure culture of the fungus in experiments 1 and 2. The range of temperature through which lesions occurred was approximately the same as that found in experiment 2. The greatest degree of severity was found at 15° and 18° C.

TABLE III.—*Effect of growing Irish Cobbler potatoes in soil inoculated with sclerotia of Corticium vagum and held at various temperatures*

EXPERIMENT 3

Temperature (° C.).	Number of sets planted.	Number of stems grown in uninoculated soil.	Stems grown in inoculated soil.						
			Total number.	Number slightly injured.	Number severely injured.	Number cut off.	Number uninjured.	Percentage injured.	Intensity of injury (points).
9	3	8	34	.....	6	5	23	32.3	79.3
11.6	3	5	41	.....	8	5	28	31.7	75.6
14.7	3	8	49	1	11	14	25	53.0	132.6
18	3	13	54	4	14	15	21	61.0	142.2
21	3	14	38	7	7	3	21	44.6	78.2
23.7	3	8	34	11	7	2	14	50.0	82.2
27	3	8	38	.....	4	.....	34	10.5	21.0
30	3	9	31	.....	.....	.....	31	.....	.....

The type of lesions produced on the stems in this experiment was identical with those obtained in the previous experiments from a pure culture of the organism. In no case were lesions found without the presence of the typical hyphae characteristic of the sterile stage *Corticium vagum*. The control plants were found to be free from both lesions and fungus.

The Early Ohio tubers used to introduce the inoculum had not passed through their dormant period. Sprouts from these made a growth of from ½ to 3 inches during the five weeks. When harvested, a number of these slow-growing stems showed distinct lesions only at the growing point. Further examination indicated that such lesions were initiated in the sinus or reentrant angle of the hook-shaped bud, at which point considerable quantities of mycelium were collected (Pl. 89, A). The

slow growth of the shoots and the protection offered in the sinus area apparently permitted the collection of hyphae and finally the killing of the bud or growing point of the stems. An explanation is thus suggested for the abundance of such localized tissue destruction at the lower temperatures where growth of the young shoot is seriously retarded. It further suggests a possible genetic relation between the growing-point injury and the lesions occurring elsewhere on the stem.

The results agree with those of experiment 2 in showing a decidedly higher percentage of diseased stems and a more severe type of lesion in the unsterilized than in the sterilized soil (Table IV and fig. 3).

TABLE IV.—*Percentage of diseased stems in sterilized and unsterilized soil*

EXPERIMENT 3

Temperature (° C.).	Unsterilized soil.	Sterilized soil.
9.1	[a] 50	12.5
11.6	50	14.2
14.7	72	33.3
18	90	34.8
21	52.3	35.8
23.7	28.9	13.3
27	23	4
30	.....	.....

[a] The water content of the unsterilized soil used in the experiment was found to be 20.4 per cent of its dry weight. No determinations were made of the change in the water relation of the soil due to sterlization.

EXPERIMENT 4.—This experiment differed from experiment 3 only in the use of Early Ohio seed. As adequate sclerotia occurred on the surface of the potatoes used for planting, the same tubers served both for an index and for the conveyance of the inoculum.

TABLE V.—*Effects of growing Early Ohio potatoes at various temperatures in soil inoculated with sclerotia of Corticium vagum*

EXPERIMENT 4

Temperature (° C.).	Number of sets planted.	Number of stems grown in uninoculated soil.	Stems grown in inoculated soil.						
			Total number.	Number slightly injured.	Number severely injured.	Number cut off.	Number uninjured.	Percentage injured.	Intensity of injury (points).
8.8	3	5	25	5	1	.....	11	24.0	28.0
11.8	3	10	33	10	5	.....	18	45.4	63.0
15.3	3	11	23	8	4	.....	11	52.1	69.6
18.2	3	12	22	3	5	2	12	45.5	86.6
20.8	3	9	24	5	2	1	16	33.3	49.7
23.7	3	9	25	7	3	.....	15	40.0	52.0
27.4	3	9	26	4	1	.....	21	19.2	22.9
30	3	9	24	.....	.....	.....	24	.....	.....

The results of this experiment, as related to temperature, agree essentially with those obtained in experiment 3 (Tables V and VI and fig. 4). The primary difference appeared in the fact that the highest percentage of diseased plants were found in the soil held at 15° C. When considered from the standpoint of total tissue destroyed, the high point apparently falls at 18°.

The percentage of infection of the Early stems was much lower and less uniform than that obtained with the Irish Cobbler stems in experiment 3.

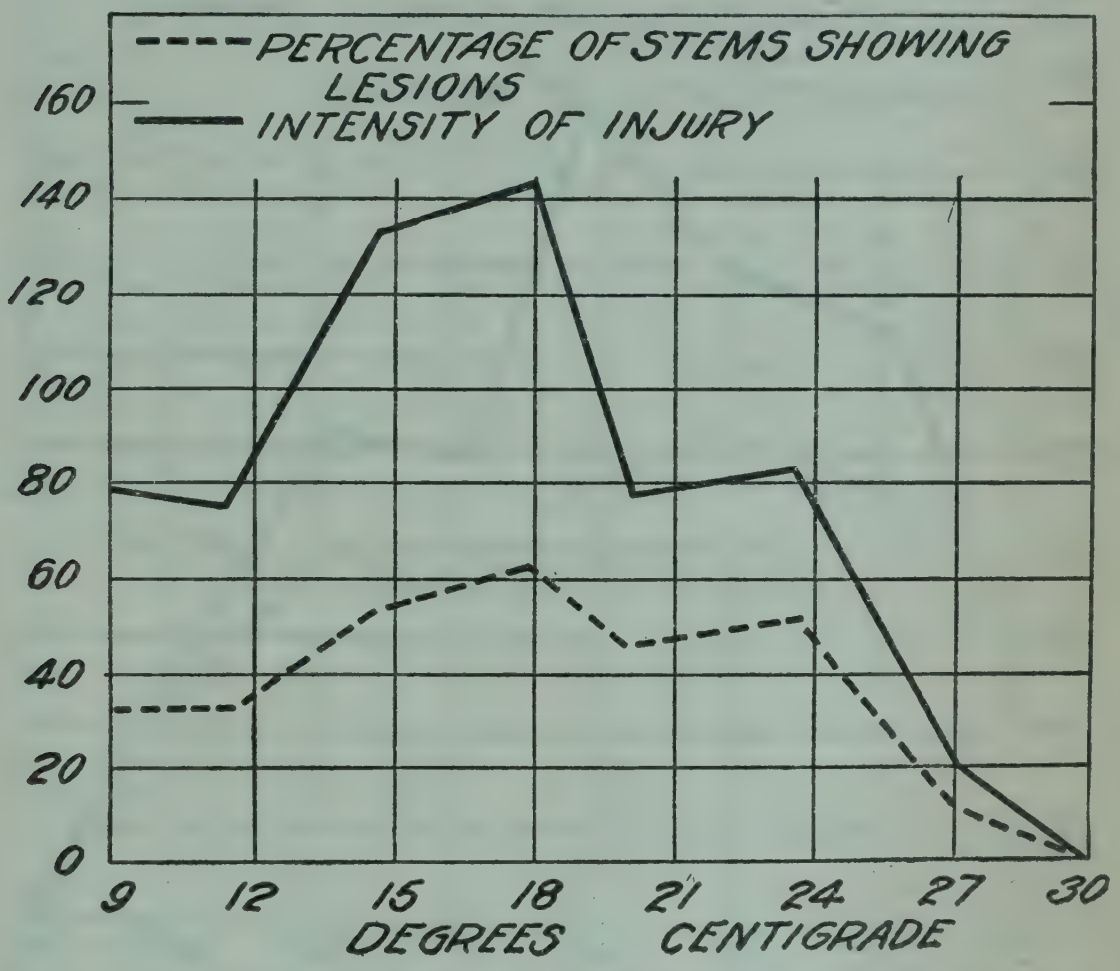

FIG. 3.—Relation of soil temperature to the severity and to the distribution of the injury caused on potato stems by *Corticium vagum* (experiment 3).

TABLE VI.—*Percentage of diseased stems in sterilized and unsterilized soil*

Temperature (° C.).	Sterilized soil.	Unsterilized soil.
8.8	30.0	30.0
11.8	26.6	61.0
15.3	22.2	71.4
18.2	.....	71.4
20.8	22.2	33.3
23.4	20.0	53.3
27.4	23.0	15.3
30	.....	.....

With the exception of the stems in one pot of sterilized soil, all control plants were found to be free from both lesions and fungus. The presence of the fungus in the one control pot resulted, apparently, from inadequate sterilization of the seed.

EXPERIMENT 5.—In this experiment, which was started at the time of early potato planting in Wisconsin, an attempt was made to approach as nearly as possible the exact conditions found in the field in normal potato culture. Surface soil was obtained directly from the field at the

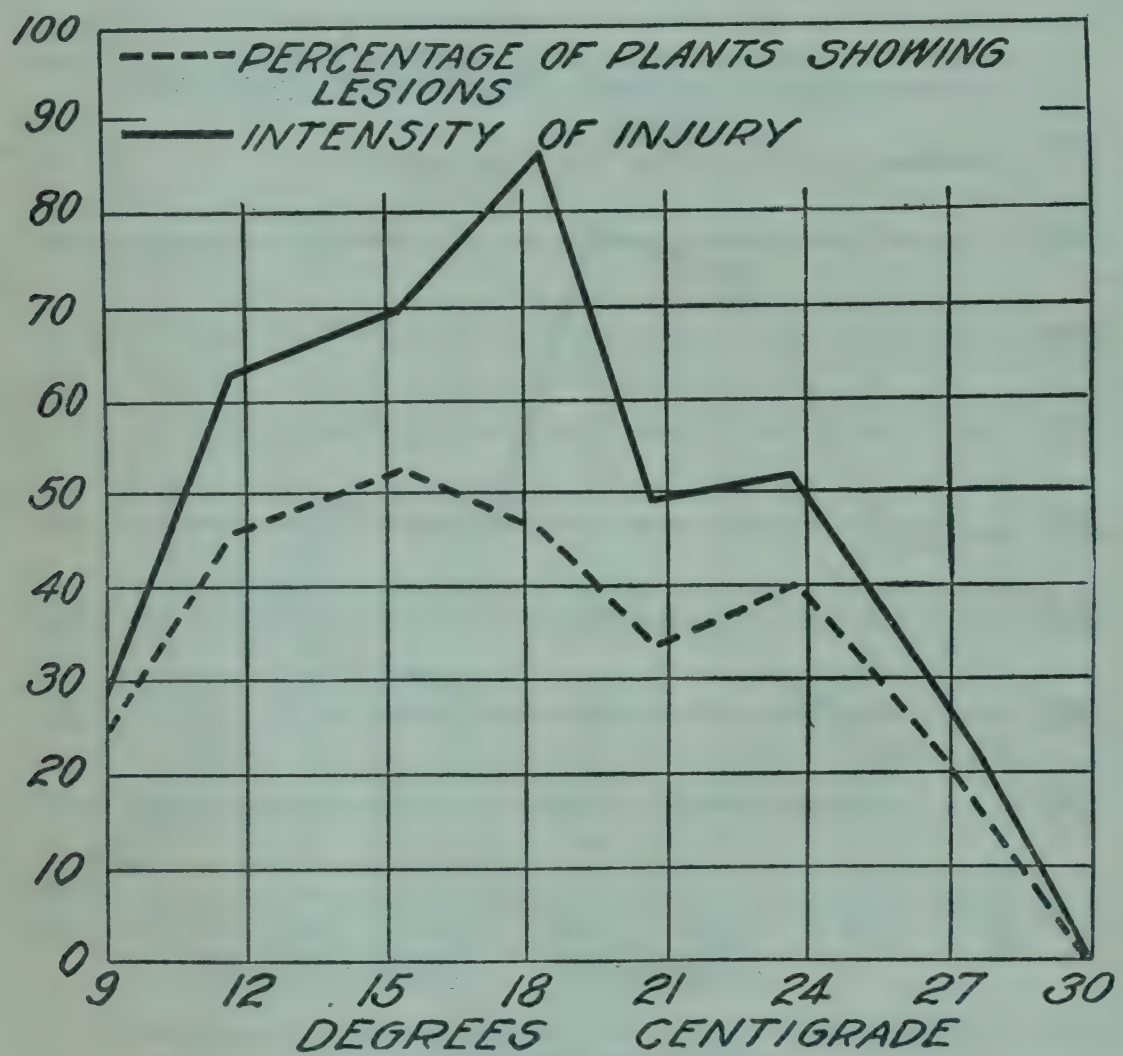

FIG. 4.—Relation of soil temperature to the severity and to the distribution of the injury caused on potato stems by *Corticium vagum* (experiment 4).

time of planting, May 17, and was placed, after mixing and without sterilization, into sterile cans. No serious modification of the water relation or the structure of the soil resulted. The soil was comparatively damp, showing a water content at the time of planting of 24.8 per cent of the dry weight.

All tubers used for seed were selected for a uniform and an abundant occurrence of sclerotia and for the absence of all bruises or scabbed areas which might function in the introduction of complicating organisms. In order to eliminate organisms loosely adhering to the surface of the

tubers used for soil inoculation, the latter were treated five minutes in a mercuric-cholrid solution (1 to 1,000). They were then washed free from the mercuric chlorid and planted. Tubers from the same lot, treated two hours in the standard solution, were planted for control. Three half tubers were planted in each can.

The plants were harvested June 15, 32 days after planting.

Under these more natural conditions lesions again occurred at from 9° to 27° C. No typical lesions were found in the soil held at 30°. The difference in intensity of injury at the various temperatures was even more distinctly marked than was found in the preceding experiments. At 24° and 27° the lesions were limited both in depth and area. Severe cortical injury resulted at 21°, and at 15° and 18° the entire cortex of many of the stems was totally destroyed (Pl. 91, A, B). Below 15° this type of critical injury again definitely decreased.

At the soil temperature of 21° C. and below, the growing points of many of the shoots were destroyed. As is shown in Table VII, this type of injury was slight at 21° but became very prominent at 18° and 15°. The highest percentage of stems showing the destruction of the primordia was found at the low temperature of 12° (Pl. 90; 91; fig. 5). In this latter respect experiment 5 differed from all previous experiments. The relation, however, was so definite as to indicate that similar results might be obtained in many cases under natural conditions in the field.

TABLE VII.—*Effects of growing Irish Cobbler potatoes at various temperatures in soil inoculated with sclerotia of Corticium vagum*

EXPERIMENT 5

Temperature (°C.).	Number of sets planted.	Number of stems grown in uninoculated soil.	Stems grown in inoculated soil.						
			Total number.[a]	Number slightly injured.	Number severely injured.	Number cut off.	Number uninjured.	Percentage injured.	Intensity of injury (points).
9.4	3	11	61	.....	8	17	36	40.9	109.6
12.2	3	11	65	3	14	31	17	73.0	190.9
15.0	3	13	62	2	16	24	20	62.4	158.6
18.2	3	8	64	4	22	23	15	76.4	182.5
21.4	3	9	51	11	20	3	17	66.6	117.5
24.4	3	16	49	13	15	.....	21	57.1	87.7
27.1	3	11	47	5	4	.....	40	19.1	27.7
30.3	3	38	47	2	1	.....	44	6.3	8.5

[a] The number of stems was determined on the basis of all stems arising from the tuber directly or as secondary shoots at or near the base of the primary stem (Pl. 90, 91). Comparatively few stems succeeded in getting through the soil at the lower temperatures.

TEMPERATURE RESPONSE OF THE POTATO PLANT IN UNINOCULATED SOIL

The available data on the temperature requirements of the potato secured by Orton (*9*, *10*) and Smith (*15*) from a study of the regional distribution of the plant and as expressed in terms of yield and general

freedom from disease must be considered as related primarily to its entire growing season. They provide no specific indications as to the relation of temperature to the various periods of the growth and development of the potato plant. So far as is known to the writer, no one has given close attention to the effect of soil temperature on growth of the potato during the first four or five weeks of its development, at which time the "Rhizoctonia" disease is most destructive.

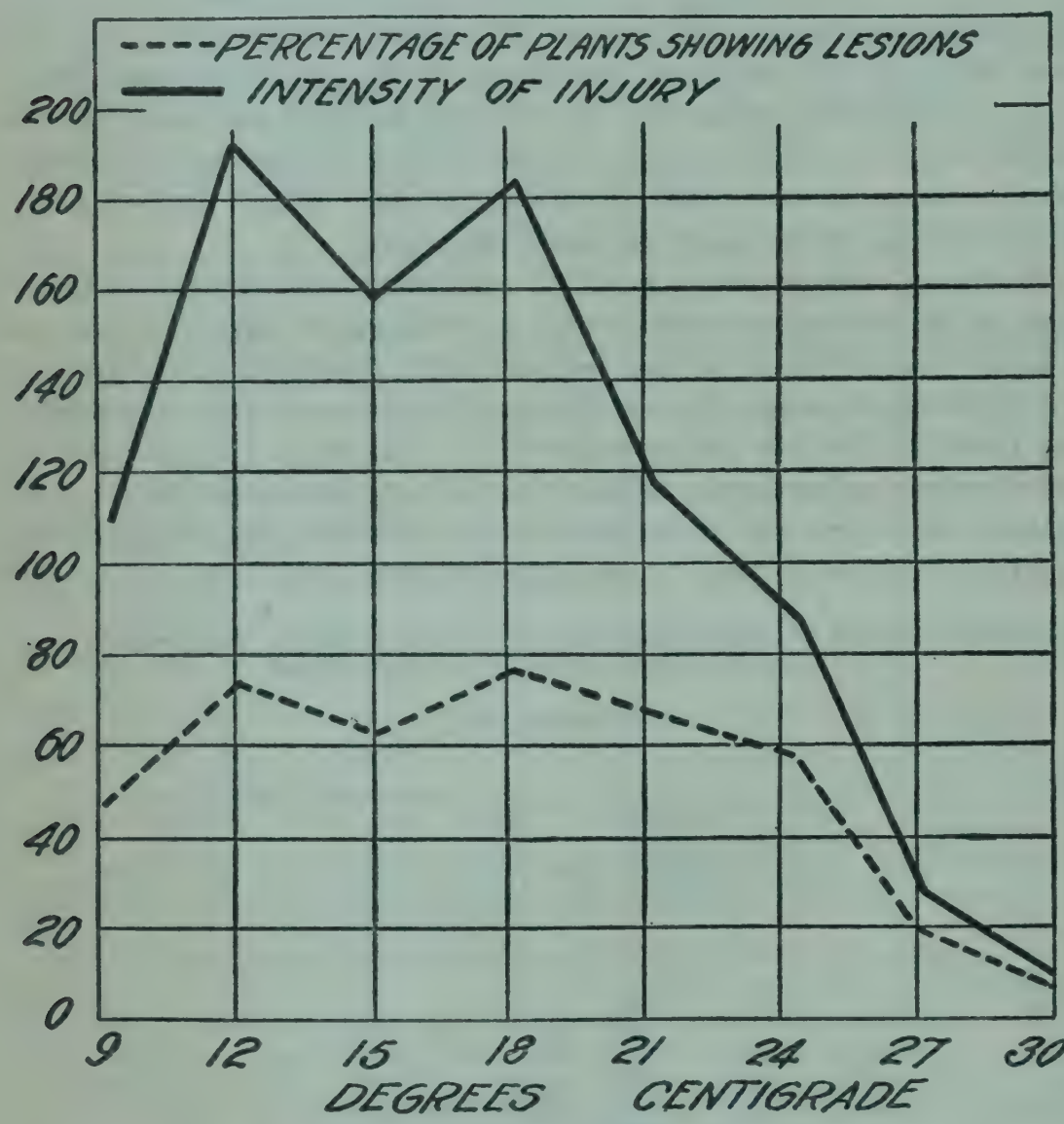

FIG. 5.—Relation of soil temperature to the severity and to the distribution of the injury caused on potato stems by *Corticium vagum* (experiment 5).

In the course of the greenhouse experiments, observations were made as to the reaction of the normal potato plant at the various soil temperatures. The data are seriously limited by the fact that such observations were of necessity confined largely to an early period of growth of a comparatively small number of control plants. The results, however, appear of sufficient interest to warrant their inclusion.

The data from the various experiments emphasized somewhat clearly the importance of the rate of growth of the young shoot during the period

in which the growing point is exposed to the fungus in the soil. This relation was determined with a fair degree of accuracy by the rate at which the plants appeared through the soil at the various temperatures. Uniformly in all the experiments the plants at 24° C. came up first. These appeared after a period of from 18 to 25 days, depending upon the vigor and state of dormancy of the tubers used for seed. In general, the plants in soil held at 18°, 21°, and 27° appeared from 2 to 5 days later than those at 24°, while those grown at 9° and 12° were delayed as much as 10 to 15 days. The plants at 30° were very irregular in their growth and in a few cases were as seriously retarded as plants at 12°.

A quantitative expression of the time estimate of the rapidity of growth is found in the data presented in Table VIII. The results are in general representative of the growth obtained in all the different experiments where adequate time was allowed before removing the plants. A decidedly different growth relation is shown at the various temperatures with the change of the seat of action from the tubers and growing points underground to the leaves and growing points above ground. The measurements obtained on December 2, 33 days after planting, together with the appearance of the plants December 15, as shown in Plate 88, B, indicates an optimum soil temperature somewhere near 18° C. for the latter periods of potato growth. This, in general, agrees with the conclusions of Orton (*9*) and Smith (*15*).

TABLE VIII.—*Effect of various soil temperatures on the rate of growth of the normal potato plant at different stages in its early development* [a]

Date.	Age of plants.	Average height of plants at various temperatures.							
		9.5° C.	12° C.	15.2° C.	18° C.	21.4° C.	24° C.	27.2° C.	30.5° C.
	Days.	*Inches.*	*Inches.*	*Inches.*	*Inches.*	*Inches.*	*Inches.*	*Inches.*	*Inches.*
Nov. 25...	25	Not up	Not up	1.4	2.1	2.7	3.2	2.5	2.0
Dec. 2....	33	Just up	2.6	4.3	5.8	5.0	4.8	3.8	3.2
Dec. 15 [b]..	46	.....	.....	.....	.....	.....	.....	.....	.....

[a] The figures represent the height of the plant above the soil.
[b] Plate 88, B, shows the relative heights of plants at this date.

The most interesting results were obtained with respect to the effect of the high temperatures upon the general morphology of the potato plant. Uniformly, at 30° C. the plants showed a decided swelling of the underground stems (Pl. 93, B). This feature was accompanied by a repression of the stolons and by what appeared to be a compensating development of leafy structures or deformed branches at the underground nodes. Excessive branching also occurred at or near the apical region of the stem just as the latter emerged through the soil. Upon further growth these branches gave a decided rosetted appearance to the plants. The aerial branches were in general very slender, with shortened internodes, and bore very small, narrow leaves which

frequently lacked the normal segmentation. These latter relations are well shown in Plate 88, A, B, and is further expressed together with other relationships in Table IX.

TABLE IX.—*Measurements on Irish Cobbler plants, grown in uninoculated soil, showing the effects of soil temperature upon the development of the various organs 33 days after planting*

	9.5° C.	12° C.[a]	15.2° C.	18° C.	21.4° C.	24° C.	27.2° C.	30.5° C.
Number of plants measured	.....	.....	6.0	5.0	9.0	6.0	9.0	5.0
Average height of shoots (in.)	.....	2.1	4.3	5.8	5.0	4.8	3.8	3.2
Average diameter of shoots (mm.)	.....	.....	7.5	8.75	7.5	7.5	6.5	5.0
Average width of leaves (mm.)	.....	.....	37.5	38.0	32.0	26.0	17.0	14.0
Number of leaves per plant	.....	.....	4.5	5.0	5.1	7.0	7.0	6.0
Average number of leaves per inch of height	.....	.....	1.0	.86	1.02	1.45	1.45	1.89

[a] At the time the data were taken the plants in the 12° C. tank were just through the soil but not sufficiently high to give reliable measurements.

At 30° C. excessive lenticle development resulted on both the underground stems of the growing plant and upon the mother tubers. This response, no doubt, appeared as results of the excessive respiration occasioned by the high temperature of the soil.

The various abnormal responses were not confined to the soil maintained at 30° C. but were found in a much less exaggerated form at 24° and 27°. Both these latter temperatures appeared decidedly unfavorable for the continued growth and development of the plant under the conditions obtained in the experiments. The plants grown at 9° and 12° showed a development which might be considered below normal and exhibited more or less spindling shoots, long internodes, few leaves, and slight yellowing. No tendency to direct tuberization was shown as was found by Vochting (*17*) to occur at 6° and 7°. On the whole, the plants showed the greatest vigor during the latter period of their early growth in the soil held at 18° and 21°. In parallel experiments which were conducted on the potato at the same temperatures and in which plants were grown approximately to maturity, the soil temperature of 15° to 18° proved more favorable than any of the higher temperatures for the plants to withstand the continued artificial conditions imposed in the experiments.

FUNGI CONCERNED IN PRODUCTION OF LESIONS

In drawing conclusions from the results obtained from the different experiments in which pure culture and sclerotial methods of soil inoculation were used, the question immediately arises as to whether in the two cases we are dealing with one and the same pathogen or strains of the same pathogen or whether in the case of sclerotia-inoculated soil

we are concerned with one or a group of organisms possibly widely different and capable under the conditions of the experiment of producing results similar to those secured with the pure culture of *Corticium vagum* described as strain 201. These questions were kept constantly in mind, and special studies were made during the progress of the work.

It is evident from the uniform freedom of the control plants from disease that the organism or organisms responsible for the lesions occurring in the inoculated soil were introduced by the various methods used and were not resident in the soil in which the potato plants were grown. In but two experiments did typical lesions occur on any control plants, and in each of these as reported the threads of "Rhizoctonia" were abundantly and unmistakably present.

The lesion on the potato stems in the sclerotia-inoculated soil were found to be indistinguishable from those produced with the pure cultures of the fungus in sterile soil. With both methods of inoculation the fungus was found constantly associated with the resulting cankers. When there was doubt the presence of the fungus was determined either by the use of the compound miscroscope or by cultural methods. During the studies isolations were also made from 138 lesions produced in sclerotia-inoculated soil. Seventy-eight per cent of these yielded pure cultures of *Corticium vagum*. From the remaining 22 per cent this fungus was obtained so intimately associated with other fungi that separation was not attempted. A somewhat higher percentage of pure cultures of the fungus was obtained from lesions produced in the artificially inoculated soil.

A further study of the casual relation of *Corticium vagum* to the lesions produced in soil inoculated with sclerotia was made by removing all the sclerotia from 25 of the potatoes selected from the same seed as was used in experiment 5. The tubers were then treated for two hours in the standard solution of mercuric chlorid and planted in contact again with the scleroti in steam-sterilized soil. The pots were then held at temperatures from 18° to 22° C. The results were comparable in every way with those obtained in the sclerotia-inoculated soil in experiment 5.

With the partially sterilized tubers in experiment 5 the same type of lesions was obtained as with the pure culture of the fungus and with untreated tuber-borne sclerotia. Finally, the temperature data in general supported the conclusion that in the two methods of inoculation the same fungus or closely related strains were responsible for the production of the type of lesions concerned. It must be kept in mind, however, that with the introduction into the soil of a potato the surface of which is covered with a large number of organisms, we are introducing complicating factors; and it seems quite probable that some of these organisms may be important factors in increasing the severity of resulting lesions, or on the other hand, they may possibly inhibit the pathogenic

action of *Corticium vagum* on the potato stems. The recent work of Edson and Shapovolov (*3*) is interesting in this connection.

From the foregoing studies and from previous work, much more extensive than outlined here, it becomes evident that the sterile stage of *Corticium vagum* is definitely capable, under natural conditions of potato culture, of producing all the primary effects on the potato plant which have been attributed to it, and that under normal field conditions other organisms, whatever they may be, are possibly of secondary or of more or less minor importance. Granting their pathogenic action under extreme conditions, we have no conclusive evidence as yet of their importance in general potato culture. It is evident, however, that this problem stands much in need of investigation.

DISCUSSION

The results obtained both with pure culture and with the natural method of soil inoculation disclosed the general fact that the organisms concerned are capable of producing lesions on potato stems over a wide range of temperature from 9° to 27° C. *Corticium vagum* becomes a serious factor in potato production, however, only at soil temperatures below 24°. While 18° proved the most favorable temperature for the pathogenic action of the fungus, it appeared evident that any wide variation in the numerous factors involved might result in a temperature requirement for maximum pathogenicity at any point between 15° and 21°. No definite critical temperature such as has been obtained for other soil organisms by Gilman (*4*), Tisdale (*16*), and Johnson and Hartman (*5*) was found to exist at the lower range of temperatures maintained in the experiments. In all cases within the range of temperature favorable for its pathogenic action *Corticium vagum* proved to be a very dangerous parasite. This range approximates very closely that found most favorable for the best development of the potato plant.

The divergence of the percentage and the intensity curves at the lower temperatures reveals a wide difference between the percentage of stems showing lesions at the various temperatures and the degree of injury to the plant subsequent to infection. This difference was found to be due both to a more severe type of tissue destruction subsequent to the initial infection at the lower temperature and to an attack on and frequent destruction of the growing point of the young shoots. This latter type of injury was seldom found at or above 21° C., while at temperatures lower than 21° it assumed decidedly serious importance.

At 18° C. the fungus exhibits a maximum of activity both in the number of stems infected and in the degree of injury subsequent to infection. The exact relation of soil temperature to the divergence between these two processes at 21° and 24° as compared with the lower temperatures is not clear. It is possible that the rapid rate of growth

of the young shoots with concomitant increased rate of cellular differentiation enters in as an important factor in lessening the degree of injury at the higher temperatures. Again, the depth and the spread of lesions may be closely associated with the growing-point injury which is most severe at the lower temperatures. On the other hand, it is very probable that decreased tissue destruction is in part at least a result of a direct inhibitive action of the higher temperature upon the physiological activities of the fungus. Balls (*2*) noted a similar decreased parasitic activity of "sore-shin fungus" (*Rhizoctonia solani*) on the cotton plant and postulated an inhibiting factor "X" in the form of a by-product of fungous metabolism. He assumes that this accumulates at the higher temperatures and limits both infection and subsequent action of the fungus on the cotton tissues. Whether or not such explanation is justified, it is evident that it does not apply to the inhibited parasitic activity which resulted in a lesser degree at temperatures below 18°. A determination of the type and the rate of enzym secretion by the fungus at the various temperatures would undoubtedly aid greatly in understanding the problem.

The relation of the temperature of the soil to the destruction of the primordia of the young shoots appears more clear. Evidence has accumulated which indicates that this type of injury depends largely upon an opportunity for the mycelium to accumulate in the sinus of the bud of the young stem in contact with the delicate primordium (Pl. 89, A). At the lower temperatures the rate of growth of the young shoots is so retarded as to increase definitely the time of exposure of the young buds to the action of the fungus in the soil. The opportunity for the accumulation of the mycelium in the growing points and for subsequent tissue destruction is thereby greatly increased.

The increase in the number of stems noted at the lower temperatures is doubtless closely associated with the growing-point destruction and must be considered as the result of a peculiar balance between the host and the parasite which is ultimately conditioned by the temperature of the soil. Granted the presence of a virulent "strain" of *Corticium vagum*, it appears evident that the number of stems which survive and finally appear through the soil in an infested area will depend to a large extent upon the duration of temperatures favorable or unfavorable for the pathogenic operation of the fungus. When a favorable temperature is maintained for a considerable period not only will large numbers of primary growing points be destroyed but secondary and even tertiary buds may succumb to the attack of the fungus, thus decreasing the number of successful stems per hill. When, however, the period of soil temperature favorable for pathogenicity of the fungus is short, resulting secondary stems may escape injury; and as two or even more may start from a single injured sprout, a decided increase in the number of stems is

made possible. On the other hand, where total absence of temperature favorable for the action of the fungus occurs, as was found in the higher temperatures, no effect on the number of stems will result.

SUMMARY

(1) The power of the sterile or "Rhizoctonia" stage of *Corticium vagum* to produce lesions on potato stems varies greatly in different localities and under varying soil and climatic conditions. A study of the factors affecting the pathogenic action of the fungus is essential to a better understanding of the disease and to the formulating of effective control measures.

(2) *Corticium vagum* causes the greatest damage to the potato at a very early stage in the development of the host, probably before the young shoots appear above the soil. Two types of stem injury are especially recognized—the cankering of the cortex of the basal portions of the stem and the destruction of the primordia of the young shoots before they push through the soil. This latter type is considered the more serious.

(3) Stem lesions produced by pure cultures of *Corticium vagum* were indistinguishable from those obtained in soil inoculated with the sclerotia of the fungus. Other fungi are recognized as possible complicating factors under natural conditions. The fungus attacked the potato stems more vigorously in unsterilized than in steam-sterilized soil.

(4) The percentage of stems injured does not give a true index to the degree of damage produced by the fungus. The intensity of injury is used to express the relative value of the different soil temperatures.

(5) The various strains of *Corticium vagum* introduced into the soil as a pure culture or as sclerotia produced lesions on the basal portion of potato stems throughout the same range of soil temperature of from 9° to 27° C. The greatest damage occurred between 15° to 21°, while 18° proved to be the most favorable temperature for tissue destruction as well as for growing-point injury. Serious destruction of tissue resulted at 9°. The severity of attack decreased rapidly above 21° until at 24° *C. vagum* proved to be of minor parasitic importance. Few typical lesions occurred at or above 27°.

(6) Growing-point destruction was confined to temperatures at or below 21°C. At 21° this type of injury was slight. On the other hand, it was found that it may reach its maximum expression at a temperature as low as 12°.

(7) Growing-point injury is dependent upon the rate at which the young shoots grow through the soil. At temperatures above 21°C. the rapid growth of the potato, together with the decreased pathogenic power of the fungus, was found to permit the primordia of the young shoots to escape uninjured.

(8) The degree of growing-point destruction appeared as an important factor in determining the increase or the decrease from the normal of the number of stems above the soil in an infected area.

(9) No "critical temperature" for the pathogenicity of the fungus on the potato appeared at the lower range of temperature used.

(10) The temperature requirements of the potato plant were found to vary with its different stages of growth. The young sprouts, while in the soil, grew most rapidly at 24° C. Growth was greatly retarded at 15° and below. At soil temperatures above 24° the plant exhibited such abnormal responses as excess branching, shortening of the internodes, decreases in segmentation of the leaves, and decrease of the diameter of the stems. A soil temperature of approximately 18° proved optimum for the later and continued development of the potato plant.

(11) The soil temperature of 18° C. found most favorable for the pathogenic action of *Corticium vagum* approximates closely the temperature of the soil optimum for the general development of the host.

(12) Soil temperature data obtained from field studies with the potato and from greenhouse experiments with the pea and the bean will be published in a later paper.

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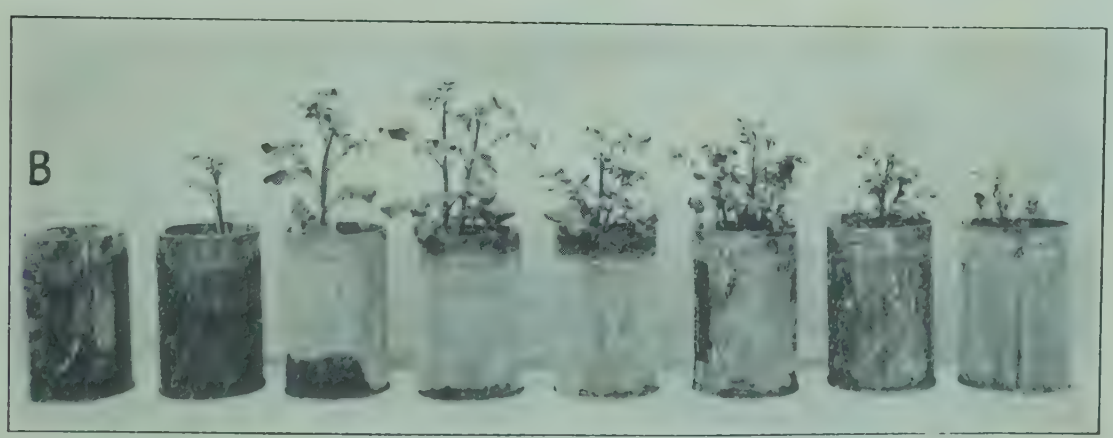
PLATE 88

A.—Irish Cobbler potato plants grown in soil held at constant temperatures of 18° and 27° C. The effect of the higher temperature is shown in the smaller size and increased number of leaves, the shorter internodes, and the decreased diameter of the stem. Comparative figures are given in Table IX.

B.—Series of Irish Cobbler potato plants grown in soil held at soil temperatures of 9°, 12°, 15°, 18°, 21°, 24°, 27°, and 30°. Plants in A were taken from a different but parallel series. Plants 46 days old.

A


B

A

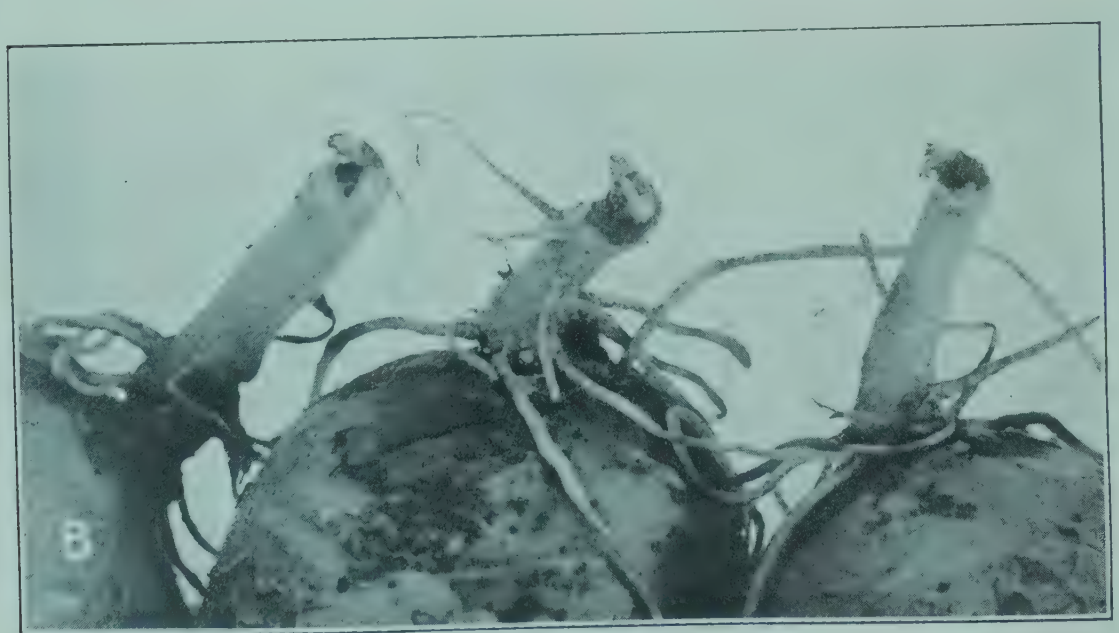

B

PLATE 89

Early Ohio potato shoots showing characteristic sinus injury. With normal seed, injury of this type occurred only at a soil temperature of 21° C. and lower. The tubers, however, from which these shoots were grown had not passed completely through their dormant period. Due to the resulting slow growth and to the increased length of exposure of the bud to the fungus in the soil, injury occurred at a temperature of 24°. (See experiment 3, p. 468–470.)

A.—Shoot showing the characteristic sinus injury and the accumulation of the mycelium of *Corticium vagum* in the sinus at the point of attack.

B.—Three additional shoots of the same seed, showing more advanced and progressive stages of the growing-point injury.

PLATE 90

Plates 90 to 93, showing plants from experiment 5, representing the type and the degree of injury to Irish Cobbler potato stems which occurred in sclerotia-inoculated soil (unsterilized) held at constant temperatures ranging from 9° to 30° C., as specified in the respective legends.

A.—Plant grown at a soil temperature of 9 °C. Twenty-seven per cent of the growing points were destroyed at this temperature.

B.—Plant grown at a soil temperature of 12°. Forty-seven per cent of the growing points of the plants held at this temperature were destroyed. Greater tissue destruction also occurred than at 9°.

1 2
A
B

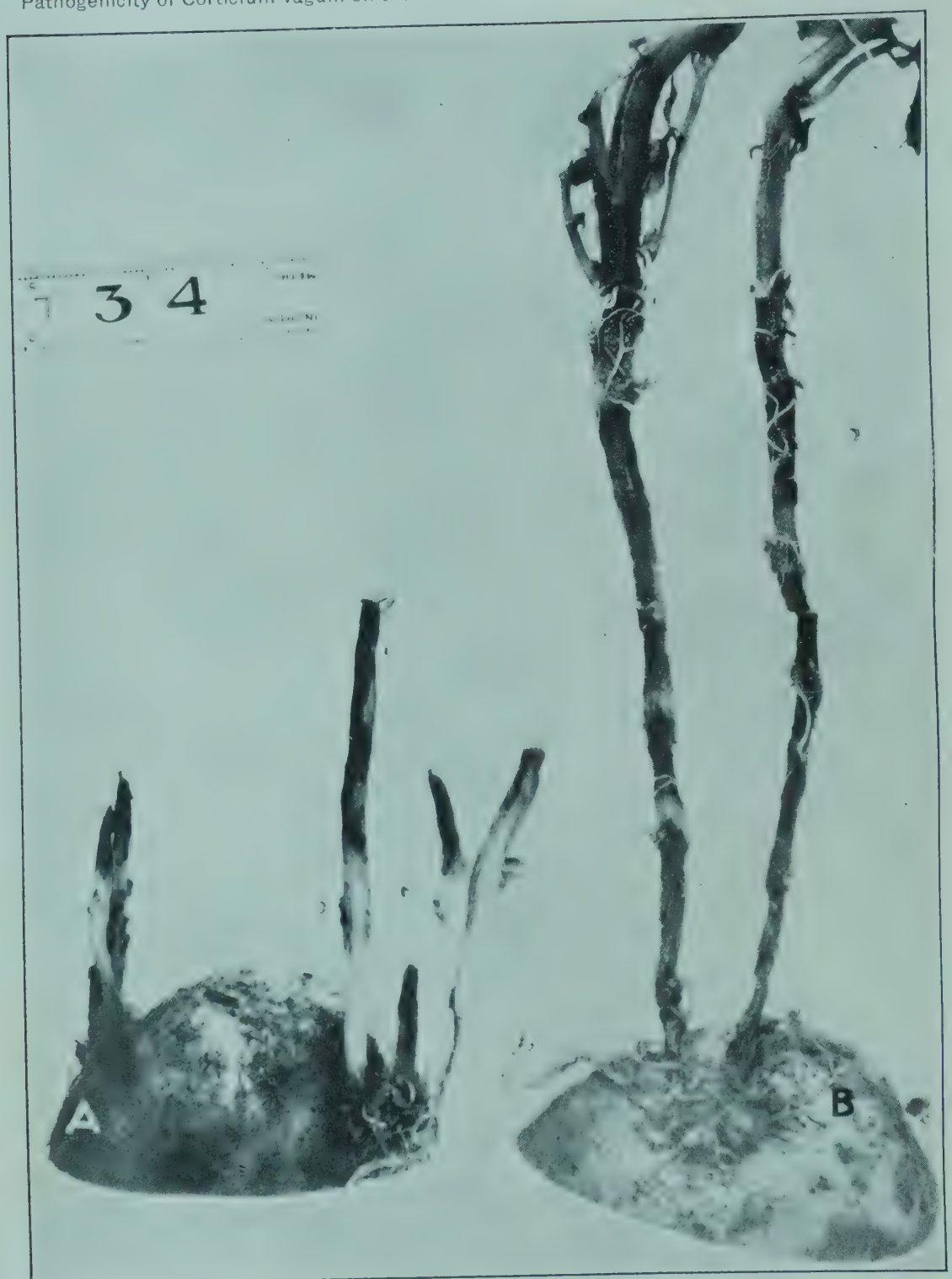

A
B

PLATE 91

A.—Plants grown at a soil temperature of 15 °C. Thirty-eight per cent of the growing points were destroyed at this temperature.

B.—Plants grown at a soil temperature of 18°. The most severe tissue destruction occurred at this temperature. Note that at only two points along the underground portion of the stem does the cortex remain uninjured. Of the five stems from this tuber, three were "cut off." Thirty per cent of the growing points were destroyed.

PLATE 92

A.—Plant grown at a temperature of 21° C. Serious cortical destruction occurred at this temperature. Only 5.8 per cent of the growing points were injured.

B.—Plant grown at a temperature of 24°. Cortical injury was decidedly less serious than was found for the lower temperatures. No growing points were destroyed.

A
B

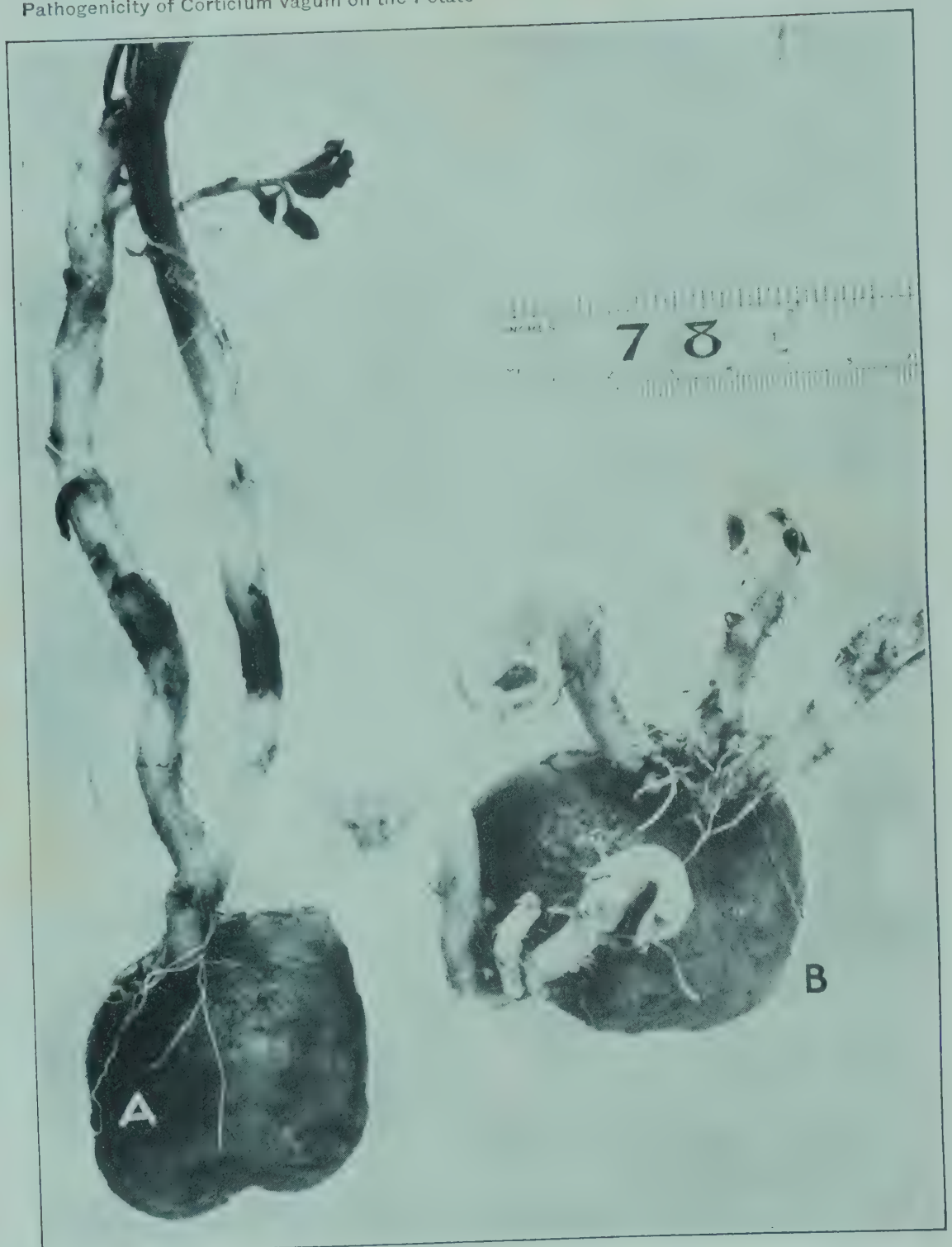

A
B

PLATE 93

A.—Plant grown at a temperature of 27 °C. Slight cortical injury occurred on 9 of the 47 plants grown in inoculated soil.

B.—Plant grown at a temperature of 30°. No typical lesions were found at this temperature.

FURTHER EXPERIMENTS IN FIELD TECHNIC IN PLOT TESTS [1]

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INTRODUCTION

In a former paper (*2*)[3] results of determinations of the effect of 18-inch alleys on the outside and inside border rows of oats, wheat, and barley varieties were made available. At that time no data appeared to be available regarding the possible border effect on rows farther within plots than the second 6-inch drill row. To secure data on this point, determinations of yield for the first, second, and third 6-inch drill rows from either side of each plot were made in 1918.

In the determinations of border effect with varieties, the rate of seeding as well as other factors were necessarily as nearly identical as it was possible to make them.

Cultural and rate of seeding tests on plots surrounded by alleys and roadways are being carried out yearly. To what extent does border effect influence results in such trials where all plots are sown with the same variety and where other conditions such as methods of preparation of the seed bed, rates of seeding, etc., are varied? In order to secure data on border effect in rate of seeding tests, determinations were made in 1918.

The data secured for the purpose of aiding in the interpretation of results in Minnesota may be of interest to scientific workers elsewhere and, therefore, are made available.

REVIEW OF LITERATURE

The need of using methods in conducting plot tests and in the interpretation of results from them which may be relied on to give close approximation to the actual results has been given considerable attention. The subject has been considered mainly from four standpoints, (1) selection of the location for the plots, (2) laying them out, which necessitates the consideration of how many repetitions, size and shape,

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[1] Published with the approval of the Director as Paper 226 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] The writer acknowledges the cooperation of Prof. T. E. Odland, formerly agronomist at the Morris Substation, and Superintendent P. E. Miller, of the same institution, for growing and caring for the crop at that location, and Prof. F. W. McGinnis, assistant professor of farm crops at University Farm, for aid in computing the results.

[3] Reference is made by number (italic) to "Literature cited," p. 497–499.

number and location of controls, alleys between plots, etc., (3) the technic of harvesting and thrashing, and (4) the interpretation of the results.

Carleton (*4*), Piper and Stevenson (*17*), Thorne (*20*), and others have considered practically the entire subject in a general way and have offered suggestions looking toward the improvement of technic in plot tests. Lyon (*12*) used original data and that of others to emphasize some of the essential considerations in plot tests.

Harris (*8*) has used the original data of Montgomery (*15*) and that of Mercer and Hall (*13*) to show the need of considering variations in the soils of fields used for experiment when interpreting the results from them. In a later article (*9*) the same writer used the original data of Kiesselbach (*11*) and that of others to further demonstrate heterogeneity of soil in fields selected for their uniformity. Emphasis is laid on the necessity for greater care in the technic of plot tests and on the use of statistical methods in the analysis of the data.

Surface and Pearl (*19*) propose a method for use in correcting for soil heterogeneity in plot tests. This method is useful only when the plots are located in blocks several in extent each way.

Smith (*18*) emphasizes the value of replication and of carrying the tests over a period of years in securing dependable results. He also mentions that the plants in one plot may have an influence on those growing in adjacent plots. Montgomery (*14*) has shown that the plants of one variety may have a considerable effect on those of another variety growing near. Hayes and Arny (*10*) report on the effect that plants in rod rows spaced a foot apart may have on each other.

That the yields of plots flanked by cultivated alleys are higher because of the effect of the additional space on the outside rows has been shown by Arny and Steinmetz (*2*). The higher yields of border rows as compared with central rows in plots flanked by alleys has been shown by Arny and Hayes (*1*), and in addition, the effect of border rows on the rank of particular varieties in tests is emphasized.

Since the publication of the earlier article (*2*) several papers which relate to this subject have come to the attention of the writer. Fletcher (*7*) reports that crops growing on the border of a fallow yielded at a rate as much as 10 times as great as the rate in the center of the plot. This is accounted for largely by the absence of toxic substances on the fallow side of the outside rows. The width of the fallow area is not mentioned.

Chittenden (*5*) gives results for turnips planted in plots 33 feet long and 7 feet 6 inches wide. The rows of turnips were 18 inches apart, and the outer rows of any two plots were 4 feet 6 inches apart. This gives 36 inches of additional space to the two outside rows flanking each alley. The rows extended east and west. For the crop planted May 25 and weighed July 29, the weights of the tops of the outer rows averaged 94 as compared with the middle row's weight of 78, both on the basis of 100 for the heaviest row. On the same basis, the roots from the outside rows aver-

aged 91 and the middle row 83. In another trial, four rows of turnips each 18 inches apart were sown July 16 in plots 13 feet by 9 feet with 9 feet 6 inches as the distance between the outside rows of any two plots. These were harvested September 28. Here on the basis of 100 for the heaviest row of each plot, the roots from the outside rows yielded at the rate of 95 and the inside rows at the rate of 70. Discarding the plants in the outside rows before estimating yield is recommended.

In a later paper the same writer (*6*) reports that potatoes in 3-foot rows, with alleys several feet wide on one side and from 4 to 5 feet wide on the ends, showed marked border effect. The rows extended north and south. Inner row No. 1, next to the outer row, yielded 72, and the third row in the plot yielded 72, as compared with 100 for the outside rows. Yields of the end plants of the rows was as 100 to 82, as compared with the average of the other plants in the same rows. In other trials end plants yielded 100 to 87, 100 to 92, and 100 to 88.4, respectively, as compared with the average for the other plants in the same rows. Corner plants—those having additional space in two directions—yielded 100 to 79, as compared with the other plants in the outside rows, and 100 to 57, as compared with the inner plants. The statement is made that in yield trials at Wisley outer rows are planted which do not essentially belong to the plots. They are to protect the rows of the plants from border effect.

Wheeler (*21*) notes that in Germany at some locations provision had been made to prevent border effect, and that in some experiments at the Rhode Island Station border effect was eliminated by removing a strip 3 feet wide on the sides and 6 feet wide on the ends. This width was decided on because the area remaining was then $^1/_{10}$ acre.

Bedford and Pickering (*3*) in field tests found the weights of the entire plants in outside rows to be heavier than the weights of the produce from the inner rows. The relation in percentage was as follows: Mustard, outside 297, 201, and 200, as compared with 100 per cent for the inner rows; wheat at Ridgemont, outside 131, inside 100; wheat at Woburn, outside 204 and 161, inside 100; barley at Harpenden, outside 126, inside 100. On plots manured from 100 to 300 tons per acre the outside rows of mustard were 190, as compared with the inner 100; on land less heavily manured the outside were 228 and the inside 100. From these results the authors conclude that, under ordinary circumstances, approximately one-fourth of the border effect is due to increased food supply and three-fourths to decrease in toxic effect.

The observation is also made that with the mustard plant the border effect did not extend beyond 6 inches from the edge of the plot, no effect in the second rows being noticed when the rows were 9 inches apart. The width of the alleys or fallow spaces between plots is not mentioned.

TECHNIC OF THE EXPERIMENT

The determinations of yield to indicate the effect of alleys on first, second, and third rows on either side of each plot were made on the wheat, oat, and barley varieties at University Farm.

A rate of seeding test for oats has been conducted at University Farm for a number of years. The rates used vary from 48 to 112 pounds per acre, which gives a wide variation in the number of seedlings per acre in the spring and in the number of culms per acre at maturity. This material served for the purpose of border effect determinations, as well as for that which it was originally outlined. The various seedings, both in the variety tests and in the rate of seeding tests, are made on the same day, as far as possible, usually in early April, and the grain is harvested during the last week in July or the first week in August.

The soil at University Farm is a Hempstead silt loam, which is not representative of any large area of the State. In order to secure a variation in soil and other environment, the rate of seeding test was duplicated at the Morris Substation, which is located on a fine silt loam of Young Gray Drift formation. This is a soil more representative of a large area of the State than that on which the University Farm is located.

There were four regularly distributed plots of each variety of oats, wheat, and barley at University Farm. In the rates of seeding tests at the Morris Substation there were 3 plots of each rate except the 96-pound rate, of which there were 5 additional used as controls. At University Farm there were 2 of each rate on 5 different methods of seedbed preparations, making 10 in all for each rate.

At Morris the plots were made up of 12 six-inch drill rows, a total width of 6 feet. The length was 130 feet. At University Farm the plots were made up of 17 six-inch drill rows, a width of 8.5 feet. For the variety test the length was 129 feet, and for the rate of seeding test 132 feet. The borders on the ends of the plots next to the roads were removed accurately before harvest by trimming to a line established by a wire stretched shortly after planting.

Alleys 1½ feet wide extended between each two plots. They were cultivated to keep them reasonably free from weeds. The variety test plots extended north and south, and the rate of seeding plots at both locations extended east and west.

At Morris the series on which the test was conducted had been in meadow the year previous. Grain crops appear to be retarded, in some instances, following meadow as compared with following corn or a grain which has followed corn. The latter part of the growing season was without rainfall, which made conditions for the grain crop still less satisfactory. The University Farm tests followed corn in regular rotations, and the rainfall during the growing season was ample.

In the variety tests three rows on either side of each plot were harvested separately, bound and tagged. They are referred to as outside border, middle border, and inside border rows, respectively. In the rates of seeding tests, two border rows were removed from either side of each plot in turn, bound and tagged. They are referred to as outside and inside border rows. In both tests the rows remaining after the various border rows were removed are referred to as central rows. They were harvested with the binder. At thrashing time each row or rows, in the case of the central areas, were thrashed with a small machine, and the grain was weighed.

The sizes of the areas from which yield determinations were made are given in Table I.

TABLE I.—*Areas from which yields were determined*

Location.	Number of 6-inch drill rows.	Dimensions of areas.	Part of an acre.
Morris Substation	1	6 inches×130 feet	1/670.15
	8	4 feet×130 feet	1/83.77
	10	5 feet×130 feet	1/67.015
	12	6 feet×130 feet	1/55.84
University Farm (rate of seeding test)	1	6 inches×132 feet	1/660
	13	6.5 feet×132 feet	1/50.77
	15	7.5 feet×132 feet	1/44
	17	8.5 feet×132 feet	1/38.82
University Farm (variety test)	1	6 inches×129 feet	1/675.35
	11	5.5 feet×129 feet	1/61.40
	13	6.5 feet×129 feet	1/51.95
	15	7.5 feet×129 feet	1/45.02
	17	8.5 feet×129 feet	1/39.73

In order to secure the yields of the various plots with no border row removed, one removed, two removed, and (for the variety test) three removed, the weights of all portions of each plot were added together to secure the yields with no border rows removed. Likewise for the variety test the weights of the central rows and the two middle border rows were added to secure the yields with the outside border rows only removed, and for the rate test the inside border rows were added. In the variety tests the weights of the central rows and those of the inside border rows were combined to secure the yields of the plots with two border rows removed. The yields for the variety plots with three border rows removed and the rates of seeding plots with two border rows removed were computed from the weights of the grain from the central rows. The total weight of grain from the central rows was divided by the number of rows in order to secure the average yield for these rows. All computations have been checked.

EFFECT OF CULTIVATED ALLEYS ON YIELDS OF DIFFERENT PORTIONS OF PLOTS IN VARIETY AND RATE OF SEEDING TESTS

Examinations of the yield data as given in Table II for the rate of seeding tests with oats grown at Morris on sod land and under comparatively dry conditions show that at each rate the outside border rows yielded very much higher (an average of 233.6 per cent) than the inside border rows, which in turn produced at a higher rate (an average of 139.7 per cent) than the central rows.

TABLE II.—*Average yields in bushels per acre of oats from rate of seeding trials harvested from border drill rows spaced 6 inches apart removed from either side of plots 6 by 130 feet at the Morris Substation and 8.5 by 132 feet at University Farm and from the central 8 and 13 rows, respectively, which remained after the removal of border rows*

Source.	Number of rows or plots.	Yield in bushels per acre and in percentage based on the yields of the central rows sown at the rate (in pounds per acre) of—					Average yield per acre.
		48	64	80	96	112	
Morris Substation:		*Bushels.*	*Bushels.*	*Bushels.*	*Bushels.*	*Bushels.*	*Bushels.*
Outside border rows	40	84.1	99.6	105.9	104.1	103.0	99.3
Inside border rows	40	56.2	59.4	68.6	58.6	53.4	59.2
Central 8 rows	20	36.8	41.2	45.8	45.4	43.4	42.5
University Farm:							
Outside border rows	100	142.9	152.4	162.9	164.1	170.7	158.6
Inside border rows	100	70.3	62.4	72.2	81.1	77.4	72.7
Central 13 rows	50	72.3	72.2	76.1	79.3	75.5	75.1
Morris Substation:		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Outside border rows	40	228.5	241.7	231.2	229.3	237.3	233.6
Inside border rows	40	152.7	144.2	149.7	129.1	123.0	139.7
Average central rows	160	100.0	100.0	100.0	100.0	100.0	100.0
University Farm:							
Outside border rows	100	197.6	211.1	214.1	206.9	226.1	211.2
Inside border rows	100	97.2	86.4	94.8	102.3	102.5	96.6
Average central rows	650	100.0	100.0	100.0	100.0	100.0	100.0

At University Farm, under more favorable growing conditions, the outside border rows yielded on an average 211.2 per cent of the yield of the central rows. The inside border rows more often yielded less rather than more than the average of the central rows. In the 64-pound rate, the abnormally low average is due to a chance combination of low-yielding rows in this particular rate of seeding.

For the work with the varieties at University Farm the data for the 1917 work are included here in order that comparisons may be more easily made. In 1918 the outside border rows yielded considerably higher than the middle, inside, or central rows for all three crops. The middle and inside border rows of the oat and wheat crops yielded practically equally, whereas for the barley crop there is a difference of approximately 5 bushels between the two yields. Both the middle and

inside border rows yielded approximately 5 bushels higher than the average for the central rows.

Considered on the percentage basis the border effect, in 1918, on the outside rows was slightly higher for the oat and wheat crops and considerably lower for the barley crop than in 1917. The most striking difference in the results for the two seasons is that in 1918 the effect of the alleys for each of the three crops is much less apparent on the second rows (the middle border rows in 1918 and the inside border rows in 1917) than it was in 1917. The average in percentage for the three crops is 115.03 in 1918 as compared with 140.93 in 1917. In 1918, the third rows from the outside yielded approximately 5 bushels more than the average for the central rows for each of the three crops. Stated in percentages, the yields of the third rows from the outside are 106.1 for the oats, 113.7 for the wheat, and 108.6 for the barley, or an average for the three crops of 109.47 as compared with the average for the central rows.

TABLE III.—*Average yields in bushels per acre of varieties of oats, wheat, and barley harvested from border drill rows, spaced 6 inches apart, removed from either side of each plot and from the central rows remaining after the removal of the border rows, and the yields of the border rows in percentages based on the yields of the central rows*

Year and source.	Oats.		Wheat.		Barley.	
	Number of rows or plots.	Average yield per acre.	Number of rows or plots.	Average yield per acre.	Number of rows or plots.	Average yield per acre.
1918:		*Bushels.*		*Bushels.*		*Bushels.*
Outside border rows	112	142.8	56	73.1	72	99.9
Middle border rows	112	82.8	56	40.8	72	60.9
Inside border rows	112	80.0	56	39.8	72	55.8
Central 11 rows	56	75.4	28	35.0	36	51.4
1917:						
Outside border rows	88	131.97	40	55.00	32	97.73
Inside border rows	88	87.95	40	40.98	32	64.56
Central 13 rows	44	71.37	20	27.25	16	42.87
1918:		*Per ct.*		*Per ct.*		*Per ct.*
Outside border rows	112	189.4	56	208.9	72	194.4
Middle border rows	112	109.8	56	116.6	72	118.5
Inside border rows	112	106.1	56	113.7	72	108.6
Central 11 rows	56	100.0	28	100.0	36	100.0
1917:						
Outside border rows	88	184.9	40	204.4	32	238.0
Inside border rows	88	123.2	40	149.3	32	150.3
Central 13 rows	44	100.0	20	100.0	16	100.0

BORDER EFFECT AND THE INTERPRETATION OF YIELDS

It has been shown that the effect of alleys increases the yields of the outside border rows very materially and, in the majority of cases, increases the yield of the second and third rows also, but to a less extent. On small plots the effect of the increased yield of these border rows

results in materially higher yields for the plots when these rows are included as compared with results when they are removed before harvest (2). These increases in yields are given in Tables IV and V.

TABLE IV.—*Comparisons of increases in yields in bushels per acre and in percentage of oats sown at different rates in pounds per acre, with no border rows removed and with one border row removed from either side of each plot, based on the yields as 100 with two border rows removed*

Location and rates of seeding (in pounds per acre.)	Yield per acre.			Increase in yield per acre.			
	No border rows removed.	Outside border rows removed.	Both outside and inside border rows removed.	No border rows removed.		One border row removed.	
	Bushels.	*Bushels.*	*Bushels.*	*Bushels.*	*Per cent.*	*Bushels.*	*Per cent.*
Morris Substation:							
48	48.0	40.7	36.8	11.2	30.2	3.9	10.6
64	54.0	44.7	41.2	12.8	31.1	3.5	8.5
80	59.9	50.4	45.8	14.1	30.7	4.6	10.0
96	58.7	49.8	45.4	13.3	29.3	3.5	7.7
112	55.0	45.1	43.4	11.6	26.8	1.7	3.9
Average	55.1	46.1	42.5	12.6	29.6	3.4	8.1
University Farm:							
48	80.4	72.0	72.3	8.1	11.2	—0.3	—0.4
64	80.5	70.9	72.2	8.3	11.5	—1.3	— .2
80	93.0	75.7	76.1	16.9	22.2	— .4	— .5
96	88.8	79.5	79.3	9.5	12.0	.2	.2
112	86.9	75.8	75.5	11.4	15.1	.3	.4
Average	85.9	74.8	75.1	10.8	14.5	— .3	— .1

TABLE V.—*Comparison of the increases in yield due to border effect of oats, wheat, and barley in bushels per acre and in percentage based on the yields with three, two, and one, border row removed in 1918, and on yields with two and one border row removed in 1917, at University Farm, St. Paul, Minn.*

Crop.	Number of border rows removed from either side of each plot.	1918							1917				
		Yield per acre.	Increase in yield per acre over that secured with—						Yield per acre.	Increase in yield per acre over that secured with—			
			One border row removed.		Two border rows removed.		Three border rows removed.			One border row removed.		Two border rows removed.	
		Bush.	*Bush.*	*P. ct.*	*Bush.*	*P. ct.*	*Bush.*	*P. ct.*	*Bush.*	*Bush.*	*P. ct.*	*Bush.*	*P. ct.*
Oats	None	83.4	.....	.....	.....	.....	.....	.....	80.5	.....	.....	.....	.....
	One	75.9	7.5	9.87	8.2	10.90	9.1	12.25	73.6	6.9	9.38	9.1	12.74
	Two	75.2	.....	.....	.7	.93	1.6	2.15	71.4	.....	.....	2.2	3.08
	Three	74.3	.....	.....	.....	.....	.9	1.20	.....	.....	.....	.....	.....
Wheat	None	40.8	.....	.....	.....	.....	.....	.....	32.5	.....	.....	.....	.....
	One	36.4	4.4	12.09	5.0	13.97	5.7	16.24	29.2	3.3	11.30	5.21	19.05
	Two	35.8	.....	.....	.6	1.68	1.3	3.70	27.3	.....	.....	1.90	6.96
	Three	35.1	.....	.....	.....	.....	.7	1.99	.....	.....	.....	.....	.....
Barley	None	58.3	.....	.....	.....	.....	.....	.....	51.4	.....	.....	.....	.....
	One	53.2	5.1	9.59	6.3	12.12	6.9	13.42	45.8	5.6	12.23	8.5	19.81
	Two	52.0	.....	.....	1.2	2.31	1.8	3.50	42.9	.....	.....	2.9	6.76
	Three	51.4	.....	.....	.....	.....	.6	1.17	.....	.....	.....	.....	.....

For the rates of seeding tests at Morris, there is a rather uniform increase in yield averaging 12.6 bushels higher with no border rows removed as compared with yields when two rows were removed from either side of each plot. At University Farm the average increase was 10.8 bushels. When the outside border rows only were removed, the yields at Morris were from 1.7 bushels to 4.6 bushels higher than when both outside and inside borders were removed. At University Farm the removal of the outside border row appears to have done away with any border effect.

In the variety test at University Farm, in 1918, inclusion of the outside rows increased the yields of the oat crop 7.5 bushels, the wheat crop 4.4 bushels, and the barley 5.1 bushels. Including the three border rows on either side of each plot increased the yields of the oats 9.1, the wheat 5.7, and the barley 6.9 bushels. In 1917, the increases in yield due to border effect were still more marked.

On farms where crops are produced on fields of considerable size, border effect is negligible on account of the relatively small proportion of the field in border. Yields reported from plots, the borders of which have not been removed are therefore probably somewhat misleading, in that they are higher than those which would be secured from large fields under the same conditions. However, the more important consideration is the effect of borders on the relative yields of the different treatments in cultural tests and of the several varieties in tests of that nature.

The yields with the standard deviations and the rank at each rate of seeding for the oats at Morris and University Farm are given in Table VI.

TABLE VI.—*Comparison of average yields per acre from plots having alleys on sides and ends with no border rows removed, with one border row removed, and with two border rows removed from either side of each plot, for various rates of seeding of oats*

Location and rate of seeding (in pounds per acre).	Number of plots.	No border rows removed.			One border row removed.			Two border rows removed.		
		Yield per acre.	Rank.	Standard deviation.	Yield per acre.	Rank.	Standard deviation.	Yield per acre.	Rank.	Standard deviation.
Morris Substation:		*Bush.*			*Bush.*			*Bush.*		
48	3	48.0	5	6.45±1.78	40.7	5	5.52±1.52	36.8	5	6.78±1.87
64	3	54.0	4	.46± .12	44.7	4	1.10± .30	41.2	4	1.84± .51
80	3	59.9	1	2.49± .69	50.4	1	.98± .26	45.8	1	1.44± .40
96	8	58.7	2	5.93±1.63	48.9	2	6.57±1.81	45.4	2	7.31±2.01
112	3	55.0	3	4.46±1.23	45.1	3	5.75±1.58	43.4	3	7.73±2.13
Average		55.1		3.96	45.9		3.98	42.5		5.02
University Farm:										
48	10	80.4	5	7.03±1.66	72.0	4	7.64±1.15	72.3	4	7.97±1.20
64	10	80.5	4	5.77± .87	70.9	5	4.22± .64	72.2	5	3.84± .52
80	10	93.0	1	7.74±1.17	75.7	3	6.20± .94	76.1	2	7.66±1.16
96	10	88.8	2	7.33±1.11	79.5	1	6.89±1.04	79.3	1	7.72±1.17
112	10	86.9	3	5.24± .79	75.8	2	4.70± .71	75.5	3	4.98± .75
Average		85.9		6.62	74.8		5.93	75.1		6.43

Inspection of the yields from the various rates of seeding at Morris, with no border rows removed, show that the 80-pound seeding gave the highest yield and the others ranked in the following order: 96 pounds, 112 pounds, 64 pounds, and 48 pounds. This rank is not changed by the removal of one or two border rows. The least significant difference between the yields of any two rates of seeding was found for this test by using the formula

$$\frac{\text{standard deviation} \times 0.6745}{\sqrt{n}},$$

where n denotes the number of plots for each rate (*22*). The result was multiplied by 3.2, which makes the odds 30 to 1 that the result is not due to chance (*16*). For no border rows removed, this figure is 4.93. Where one and two border rows were removed, the figures are 4.96 and 6.27, respectively. If these are used in a broad way in the interpretation of the results, it appears that where no border rows were removed the 80-pound rate of seeding gave better results than the 64-pound rate, which in turn gave better results than the 48-pound rate. The interpretation of the results where one and two border rows were removed would not be much different.

At University Farm the rank with one and two border rows removed tends to be somewhat different than with no borders removed. Employing in a broad way 4.51, 4.06, and 4.38 bushels, respectively, as the least significant differences where none, one, and two border rows were removed, an interpretation of the results may be made. Where no border rows were removed, the 80-pound rate appears to have yielded the highest, with the 96-pound and 112-pound rates equal and in turn higher than the two lower rates. For the tests with one and two border rows removed, the 96-pound rate seems to have maintained a lead over the 80- and 112-pound rate and to have yielded significantly higher than the 64- or 48-pound rate of seeding.

The results for the variety tests of oats, wheat, and barley in 1918 with none, one, two, and three border rows removed are given in Table VII.

For the oat and barley varieties there are some changes in rank brought about by the removal of border rows. It is of interest to note that the removal of borders makes no change in the rank of the last four varieties, which were also the lowest in rank in the 1917 tests. In the barley variety test the rank of the Manchuria cross and the Chevalier are not changed. The Chevalier ranked the highest in 1917.

With the same formula as was used in the rate of seeding tests to secure figures representing the least significant differences, the following were secured for the oat tests: No borders removed, 5.26 bushels; one removed, 4.54 bushels; two removed, 4.46 bushels; and three removed, 6.90 bushels. These amounts have been subtracted from the

highest yielders under each method of test, and lines have been drawn at what may be considered the discard point for the season. Removal of one and three border rows, respectively, from either side of each plot lowers the discard point in the test. Due to the removal of the border rows, there are a number of changes in rank; but in general the varieties above the discard point with no border rows removed remain there under the other methods of test, except as has been previously noted.

For the wheat variety test the least significant differences in bushels per acre for the various methods of test are as follows: No borders removed, 1.67; one removed, 1.71; two removed, 1.92; three removed, 2.14. Lines have been drawn at what may be considered the discard point for the season. When no border rows were removed, Marquis gave a lower yield than Mindum or Preston; but with one border row removed from either side of each plot, Marquis equaled the other two in yield and maintained that rank with two and three border rows removed.

In the barley variety test the least significant differences were found to be for no border rows removed, 3.94 bushels; one row removed, 3.62 bushels; two rows removed, 3.58 bushels; and three rows removed, 3.97 bushels.

The removal of one border row from either side of each plot put the yield of one variety below the discard point adopted. The removal of three border rows put Svansota below the discard point. Removal of two border rows also raised Improved Manchuria from a rank of 6 to a rank of 4 in yield.

TABLE VII.—*Comparison of average yields per acre for 1918, for four $\frac{1}{40}$-acre plots (approximate size) with no border rows removed, and with one, two, and three border rows removed from either side of each plot for 14 varieties of oats, 7 varieties of wheat, and 9 varieties of barley*

Crop and variety.	Accession number.	Descriptive note.	No border rows removed.			One border row removed.			Two border rows removed.			Three border rows removed.		
			Yield per acre.	Standard deviation.	Rank.	Yield per acre.	Standard deviation.	Rank.	Yield per acre.	Standard deviation.	Rank.	Yield per acre.	Standard deviation.	Rank.
			Bushels.			*Bushels.*			*Bushels.*			*Bushels.*		
OATS.		Time of maturity:												
Lincoln	505	Medium	91.5	4.12±0.98	1	82.2	3.43±0.81	1	81.9	3.82±0.91	1	80.3	3.38±0.81	1
Selection [a]	358	.....do	90.2	6.55±1.56	2	81.8	5.36±1.28	2	79.8	5.33±1.27	2	77.2	6.28±1.50	6
Improved Ligowa [a]	281	.....do	88.8	8.16±1.95	3	80.1	7.67±1.83	3	78.8	8.43±2.01	4	78.7	9.31±2.22	3
Victory	314	.....do	88.1	1.59± .37	4	79.8	1.70± .41	4	79.0	2.07± .49	3	78.9	2.12± .51	2
Minota [a]	512	Medium early	87.7	8.48±2.02	5	79.1	8.75±2.09	6	78.3	9.04±2.16	5	78.1	9.27±2.21	4
Silvermine	506	Medium	86.9	3.84± .96	6	78.7	3.67± .88	7	79.8	1.08± .26	2	77.0	4.08± .97	7
Do. [a]	337	.....do	86.6	2.86± .68	7	79.4	3.00± .72	5	78.1	2.59± .62	6	77.4	2.56± .61	5
O. A. C. 72	500	.....do	[b] 86.1	2.05± .49	8	77.9	2.21± .53	8	[b] 77.3	1.42± .34	7	76.2	1.85± .44	9
Banner	507	.....do	85.7	3.19± .76	9	[b] 77.5	2.55± .61	9	76.9	2.92± .70	8	76.7	3.18± .76	8
Swedish Select	502	.....do	84.1	5.23±1.25	10	76.1	4.92±1.17	10	74.9	5.36±1.28	9	[b] 73.6	5.42±1.29	10
White Tartar	339	Late	76.8	8.53±2.03	11	69.8	2.42± .58	11	69.2	3.20± .76	10	68.4	2.93± .70	11
Iowa 103	531	Early	73.2	4.64±1.11	12	68.2	4.33±1.03	12	67.5	3.95± .94	11	67.3	4.17± .99	12
Kherson	261	.....do	71.6	5.64±1.34	13	66.2	5.76±1.37	13	66.2	5.50±1.31	12	65.9	5.92±1.41	13
O. A. C. 3	491	.....do	70.9	3.32± .79	14	66.2	3.14± .75	14	65.5	3.10± .73	13	64.9	3.44± .82	14
Average			83.4	4.87		75.9	4.21		75.2	4.13		74.3	6.39	
WHEAT.		Type:												$
Mindum [a]	470	Durum	45.8	2.30±0.55	1	41.0	2.16±0.52	1	39.8	1.97±0.47	1	38.6	2.40±0.57	1
Preston [a]	924	*vulgare*	[b] 45.0	1.90± .45	2	39.6	1.94± .46	2	39.7	2.14± .51	2	38.2	2.44± .58	2
Marquis	1239	.....do	43.2	1.43± .34	3	[b] 39.2	1.93± .46	3	[b] 38.1	2.21± .53	3	[b] 37.7	2.35± .56	3
Preston×Preston [a]	188	.....do	41.5	0.16± .04	4	36.8	0.02± .00	4	35.7	0.50± .12	4	35.0	0.45± .11	4
Glyndon Fife [a]	163	.....do	38.7	1.96± .47	5	34.4	1.76± .46	5	34.2	1.81± .43	5	34.2	2.11± .50	5
Haynes Bluestem [a]	169	.....do	36.0	1.25± .30	6	32.4	1.43± .34	6	32.0	1.56± .37	6	32.0	1.68± .40	6
Acme	1967	Durum	35.2	1.84± .44	7	31.6	1.83± .44	7	30.9	2.26± .54	7	30.1	2.42± .58	7
Average			40.8	1.55		36.4	1.58		35.8	1.78		35.1	1.98	

BARLEY.		Type:															
Lion×Manchuria [a]	438	6-row smooth awn	64.3	4.35±0.10	1	60.0	4.02±0.10	1	58.9	3.76±0.90	1	58.8	4.00±0.95	::
Do	437	do	62.9	3.35±.08	2	59.3	3.25±.08	2	58.5	3.52±.83	2	[b] 57.6	3.68±.88	2
Svansota [a]	440	2-row *erectum*	61.0	6.15±.15	3	[b] 56.5	4.92±.12	3	[b] 55.3	4.93±1.18	3	53.9	4.63±1.10	3
Manchuria [a]	105	6-row common	[b] 60.7	1.66±.04	4	53.9	5.52±.13	4	50.7	1.13±.27	6	49.5	1.41±.34	7
Minsturdi [a]	439	True 6-row	59.3	5.78±.14	5	53.8	2.66±.06	5	52.1	5.20±1.24	5	51.3	7.37±1.76	6
Improved Manchuria [a]	184	6-row common	58.7	2.12±.05	6	52.9	1.70±.04	6	52.5	2.94±.70	4	52.5	3.31±.79	4
Manchuria×Manchuria [a]	390	do	57.2	2.65±.06	7	52.3	1.25±.03	7	52.1	2.08±.50	5	52.2	2.31±.55	5
Do [a]	388	do	45.0	4.38±.10	8	48.2	4.18±.10	8	46.6	4.02±1.00	7	46.1	4.19±1.00	8
Chevalier [a]	230	2-row *nutans*	46.4	2.43±.06	9	42.2	2.62±.06	9	41.5	2.30±.55	8	41.0	2.24±.55	9
Average			58.3	3.65		53.2	3.35		52.0	3.32		51.4	3.68	

[a] From the Plant Breeding Section, University Farm.

[b] Discard point for year.

SUMMARY AND DISCUSSION OF RESULTS

From the data given in Tables II and III, it appears that, subject to the influence of environment and varying somewhat with the crop, the effect of 18-inch cultivated alleys on the outside rows of plots is quite uniform and marked.

The effect on the second 6-inch drill rows within the border of the plots appears to be more variable. Thus, as shown in Table III, in the 1917 variety test, the effect ranged from 123.2 per cent in oats to 149.3 per cent in wheat and 150.3 per cent in barley, based on the average yields of the central rows. In the 1918 variety test, the second rows averaged 109.8 per cent for oats, 116.6 per cent for wheat, and 118.5 per cent for barley, based on the yields of the central rows. Considering the results for the rate of seeding tests at Morris, the second rows, inside border rows, yielded at the rate of from 152.7 per cent for the thinnest seeding to 123.0 per cent for the thickest, based on the yields of the central rows. The greatest border effect on the second rows appears to be in the thinnest-sown, and the least effect in the thickest-sown plots. In the rate of seeding test at University Farm there appears to have been no effect on the second rows.

The effect on the third rows, inside border rows of the 1918 test, was slightly less than the effect on the second rows for each crop. They yielded at the rate of 106.1 per cent for oats, 113.7 per cent for wheat, and 108.6 per cent for barley, based on the yields for the central rows as 100.

Therefore, in this test with varieties of oats, wheat, and barley, the influence of 18-inch cultivated alleys extended to the third 6-inch drill rows within the plots on either side. When sown at this distance apart, the third drill rows occur at a distance 15 inches from the outside boundaries of the plots. Possibly the differences in the crops grown and in the environment may account for the influence of the borders being exerted to a greater distance within the plots than was observed by Bedford and Pickering (*3*). In their tests it is not stated whether a crop was growing on the other side of an alley or whether the rows were bordered by a considerable fallow space. A considerable fallow area presumably would exert an influence farther within a plot than a narrow cultivated alley flanked on the other side by the same crop.

Bedford and Pickering (*3*) found it necessary to make the outside rows, since the actual outside rows were often found to be so badly injured by the cultivating implements as to be unfit for use. In the absence of any statement on this matter, it is presumed that the rows of plants were removed from the plots very early before the root systems had become moderately well established. Otherwise the border effect may have been modified considerably by the growth of the plants before removal and later by the presence of the decaying roots in the soil.

It is not within the scope of this paper to discuss in any detail the reasons for border effect; but, from the greater effect in the thickness of seeding test at Morris in 1918 under comparatively dry conditions both on the first and second rows in the plots as compared with the results at University Farm, where favorable moisture conditions prevailed, it seems reasonable to assume that increased moisture supply with accompanying results probably is one of the prominent factors.

As to the effect of border rows on the interpretation of results, it has been shown that (1) where they are not removed the yields of plots are higher than where these rows are eliminated before harvest and (2) the rank of a variety or of a rate of seeding in relation to the discard point derived for the particular test may change on account of the removal of border rows.

If the only effect of border rows lay in increasing yields beyond what would be secured in large fields under like conditions, the expense of removal of borders would probably not be warranted. However, when the interpretation of the results, as has been brought out in Tables VI and VII and in the results for the 1917 variety tests (*2*), is necessarily different in some instances when the yields with the border rows is considered than when they are removed, the matter warrants very careful consideration. It is of interest to note in this connection that the removal of the border rows did not necessarily reduce the probable error in the tests.

When border rows are removed, the question arises as to how many. From the 1917 results (*2*) it seemed desirable to remove two border rows. In the 1918 tests, the effect on the second rows at Morris is very marked and in this respect is similar to the results with the varieties at University Farm in 1917. At University Farm, the effect on the second rows in the variety tests in 1918 is less marked than in the same tests in 1917, but the effect is shown to extend definitely to the third rows. In the rate of seeding test with oats there was practically no effect on the second rows.

From the data given, unless border effect can be prevented in some other way, it appears advisable as a precaution to insure the most reliable results to remove at least two 6-inch border rows from either side of grain plots bounded by alleys or roadways.

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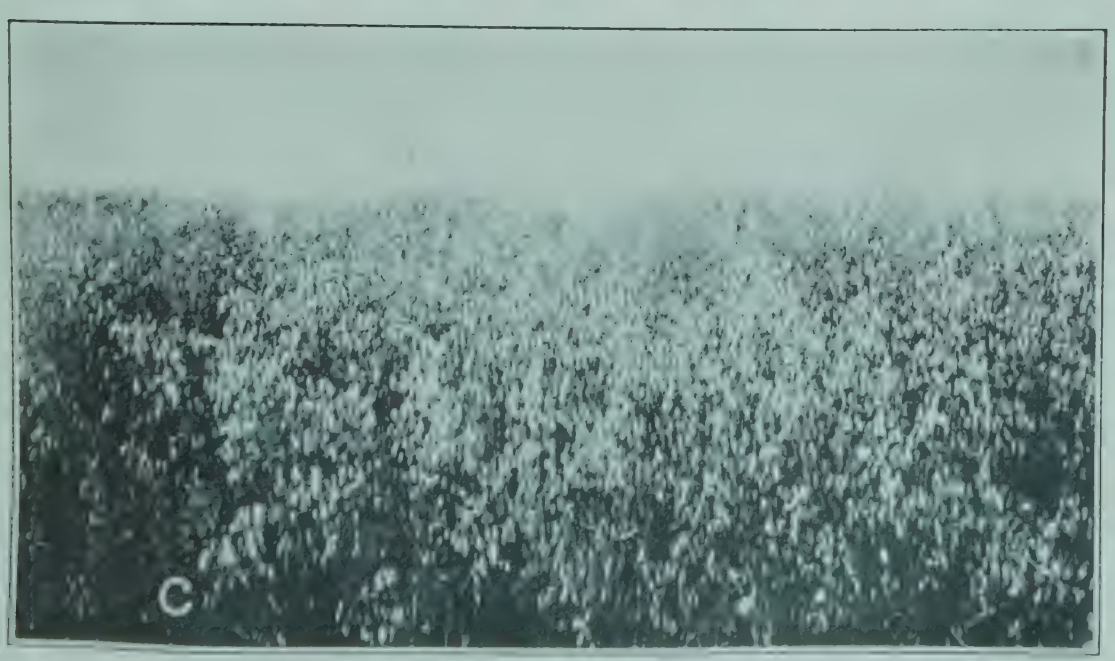
PLATE 94

Border effect shown in the experiment on rate of seeding of oats at Morris in 1918.
A.—Oats sown at rate of 48 pounds per acre.
B.—Oats sown at rate of 96 pounds per acre.
C.—Oats sown at rate of 112 pounds per acre.

A

B


C

RELATION OF HORSE NETTLE (SOLANUM CAROLINENSE) TO LEAFSPOT OF TOMATO (SEPTORIA LYCOPERSICI)

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INTRODUCTION

Horse nettle (*Solanum carolinense* L.), a weed closely related to potato and tomato and generally distributed in fields and waste places from Ontario to Massachusetts, Florida, Illinois, Nebraska, and Texas, has been found to be a host of the tomato leafspot, or blight, fungus.

In Maryland, New Jersey, and Delaware, where tomato leafspot causes heavy annual losses, horse nettle (Pl. 95, 96) is very common in waste places, in grass and grain fields, and even in cultivated crops. Its numbers in fields of tomatoes in the Middle Atlantic States, especially in Delaware, not infrequently exceed those of the tomato plants 5 to 20 times. Moreover, it bears numerous spots (Pl. 97, A) which in size, shape, color, and other appearances closely resemble leafspot of tomato (Pl. 98, A). Observations of these facts caused the writers to determine whether or not horse nettle is a host of the tomato leafspot fungus.

EXPERIMENTAL WORK

MATERIAL AND METHODS

Young tomato and horse nettle plants were grown from seed in the greenhouse and used for inoculation when 1 to 5 inches high. Considerable difficulty was at first encountered in germinating the horse nettle seed, but this was finally overcome by setting the seed pots directly on the steam pipes. Both the tomato and horse nettle seedlings were in a thrifty condition when inoculated. Control plants were used in approximately as large numbers as inoculated plants in each experiment.

The inoculations were made by spraying the plants with spores from a pure culture by means of either a DeVilbiss atomizer or a small spray pump, or by transferring minute pieces of mycelium from a rapidly growing culture to leaves moistened with distilled water. Both the inoculated plants and the controls were kept in a moist chamber 48 to 60 hours and then transferred to benches in the greenhouse.

INOCULATION OF HORSE NETTLE PLANTS WITH SEPTORIA LYCOPERSICI FROM TOMATO

In the fall of 1919 about 25 horse nettle plants transferred from the field to the greenhouse and inoculated with a pure culture of *Septoria lycopersici* Speg obtained from tomato became infected with numerous

spots resembling leafspot of tomato, while an equal number of horse nettle plants not inoculated but kept in the same environment as the inoculated plants remained healthy. During the winter of 1919–20 more than 500 horse nettle seedlings 1 to 5 inches high were inoculated with this fungus, and as many more were used as controls. The leaves of about 90 per cent of the inoculated plants became infected, but not one of the control plants showed any symptoms of the disease.

The culture used in making these inoculations was isolated from a Septoria spot on a tomato leaf and had been used to infect several thousand tomato seedlings. In morphology, in growth on standard culture media, and in development on tomato leaves it was typical for *Septoria lycopersici*.

The horse nettle leafspots (Pl. 99, A) when small were usually somewhat circular in outline and brown in color, but when older they became more irregular in outline, light-colored in the center and dark at the margins. There is a larger percentage of circular spots in Plate 99, A (artificially infected leaves) than in Plate 97, A (naturally infected leaves); but this is due largely, if not wholly, to the fact that the spots in Plate 99, A, are younger than those in Plate 97, A. The larger spots in both plates are more or less irregular in outline. In fact, the shape of the spots on tomato leaves (Pl. 98, A) varies from a circular form when small to a more or less irregular outline when large.

The horse nettle seedlings were not quite so susceptible to the leafspot fungus (*Septoria lycopersici*) as tomato seedlings. They showed more individual variation in resistance but became infected readily and became fairly thickly covered with spots (Pl. 99, A).

The spots on the horse nettle leaves infected in the greenhouse differed somewhat from those on the tomato leaves in pycnidia production. Pycnidia appeared on nearly all the spots on tomato leaves but only on a relatively small percentage of the spots on horse nettle leaves. They were usually numerous on this small percentage of horse nettle spots but were more deeply imbedded than on tomato leaves and were not always easily seen without the aid of a hand lens. The appearance of both the spots and pycnidia on tomato and horse nettle leaves may be seen in Plates 97, B; 98, B; 99, B, C.

The pycnidia on the horse nettle leaves produced innumerable spores which where indistinguishable from those taken from tomato leaves. Both the pycnidia and spores in mass are shown in a section of horse nettle leaf in Plate 99, B, C. Many of the pycnidia were even more deeply imbedded than the pycnidium shown in Plate 99, B, which in surface view was not very conspicuous to the unaided eye.

Since pycnidia of *Septoria lycopersici* developed rather sparingly on horse nettle leaves in the greenhouse, 222 horse nettle plants inoculated with a culture of *S. lycopersici* obtained from tomato were planted outdoors where the air was supposedly drier; but only a few scattered pycnidia developed on these plants until nearly the end of the growing

season. They were then found chiefly in small, dark brown spots lying in large dead areas near the tips and edges of the leaves. As pycnidia did not appear on tomato leaves in the field until very late in the fall, it is quite possible that the very wet weather during August hindered their development on both tomato and horse nettle. In fact it has been repeatedly noted while working with this fungus, both in the greenhouse and in the field, that the development of pycnidia on tomato plants is favored by dry air and hindered by moist air. This variability in sporulation occurs also on culture media. The drier areas in corn meal cultures of *S. lycopersici* develop pycnidia readily, while those that are moist produce them only after the medium becomes somewhat dry or not at all.

INOCULATION OF TOMATO AND HORSE NETTLE SEEDLINGS WITH SEPTORIA LYCOPERSICI REISOLATED FROM HORSE NETTLE LEAVES

Thirty young tomato plants and 14 young horse nettle plants 3 to 6 inches tall were inoculated by spraying their leaves with spores of a culture of *Septoria lycopersici* reisolated from horse nettle. Twenty uninoculated tomato plants were sprayed with distilled water and used as controls. Both the inoculated plants and the control plants were kept in a moist chamber 60 hours.

All the tomato and horse nettle plants became heavily infected, but none of the controls developed any symptoms of the disease. Pycnidia developed on the spots, but they were much more common and more conspicuous on the tomato than on the horse nettle. The spores, which developed in profusion, were identical in shape and size with those produced by the original culture obtained from tomato.

SEPTORIA ON NATURALLY INFECTED HORSE NETTLE

Spots resembling leafspot of tomato began to appear on horse nettle leaves at the Arlington Experimental Farm and in fields and gardens in the vicinity of Washington about the middle of August but bore very few if any pycnidia before the middle of September. This was approximately coincident with the appearance of pycnidia of *Septoria lycopersici* on tomato leaves.

The pycnidia found on naturally infected horse nettle leaves after the middle of September were rather numerous but appeared on only about 1 per cent of the spots. They contained innumerable spores that were indistinguishable morphologically from those of the tomato leafspot fungus, *Septoria lycopersici*. Dead areas near the tips and margins of the leaves seemed to afford the most favorable conditions for their development, for they were found most frequently within these areas.

Several attempts were made to isolate a pure culture of the Septoria from naturally infected horse nettle leaves by use of the spores, but in each case the spores failed to germinate. The writers were not surprised at these results, however, as it often requires repeated efforts to isolate

a culture of *Septoria lycopersici* from tomato leaves even when spores are used. Moreover, it is still more difficult to isolate Septoria from minute pieces of infected tissue, such as may be obtained from spots on tomato leaves, because these infected tissues are nearly always invaded by other organisms, and a treatment that will kill these accompanying organisms will kill Septoria. As Septoria grows very slowly in culture other organisms, if present, soon prevent its development by growing over the surface of the culture medium. It is very likely that Septoria could have been isolated from these naturally infected horse nettle leaves about as easily as from tomato leaves, however, had not a frost defoliated the plants when the plating of the spores was started and made it almost impossible to find more spore-bearing material.

ISOLATION OF ORGANISMS OTHER THAN SEPTORIA FROM LEAFSPOT OF HORSE NETTLE

While looking for pycnidia of Septoria on naturally infected horse nettle leaves, the writers observed a Phoma, an Alternaria, and two species of Cladosporium fruiting on the surface of the dead spots. One Cladosporium resembled *Cladosporium fulvum* Cke. in the shape of its spores and in the production of a purple color on both cornmeal and oatmeal agar but differed from it in the production of smaller spores.

The pathogenicity of each of these fungi was tested in four to five series of experiments by inoculating with a pure culture thrifty young tomato and horse nettle seedlings and keeping them in a moist chamber 48 to 60 hours after inoculation. No spots resembling those occurring naturally on horse nettle or those produced by spraying horse nettle or tomato plants with spores of *Septoria lycopersici* developed on any of these plants. Seven out of 100 tomato plants inoculated with the Alternaria developed an infection spot, but it was an elongated area following the veins and was found also on a few uninoculated plants of the same age on the greenhouse benches. Moreover, it did not appear on horse nettle. It would therefore seem that the four fungi isolated from horse nettle leafspots were merely saprophytes.

DISCUSSION

Septoria lycopersici is a very active parasite. It readily infects all the varieties of tomatoes grown in the United States and all varieties, both wild and cultivated, that the writers have been able to obtain through the Office of Seed and Plant Introduction. It infects very easily *Cyphomandra betacea* Sendt., the tree tomato, and a species of Solanum from Ecuador, *Solanum mammosum* L. On this Solanum it also produces numerous pycnidia and spores. It is not surprising, therefore, that it infects *Solanum carolinense*.

Since *Septoria lycopersici* in pure culture infected horse nettle seedlings readily and developed pycnidia and spores both in the greenhouse and outdoors, it is apparently able to maintain itself on this host. The

Septoria pycnidia found on naturally infected horse nettle were probably also those of *S. lycopersici*, since they were similar to them in size, form, and spore contents and developed on approximately the same percentage of spots as the pycnidia on the artificially infected horse nettle plants.

It is very likely that in the Middle Atlantic States most if not all the horse nettle leafspots resembling tomato leafspot are caused by *Septoria lycospersici*. The fact that *S. lycopersici* infects horse nettle easily and produces spots, pycnidia, and spores that can not be distinguished morphologically from those occurring on horse nettle in the field would tend to support this conclusion. Moreover, it would also seem to be strengthened by the failure of the other organisms isolated from these spots to reproduce the leafspot disease.

The results of the inoculations with a pure culture of *Septoria lycopersici* show that horse nettle is a very susceptible host for this parasite. It should therefore be kept out of prospective tomato fields to prevent its harboring the tomato leafspot fungus and thereby largely destroying the benefits that would otherwise be secured from rotation of crops. For the same reason it should be destroyed in fence rows, roadsides, and other waste places near tomato fields

SUMMARY

Horse nettle, a weed common in fields and waste places in the eastern half of the United States, usually bears in the Middle Atlantic States numerous spots on its leaves resembling leafspot of tomato. Inoculation of more than 500 horse nettle seedlings with a pure culture of the tomato leafspot fungus, *Septoria lycopersici*, caused infection of about 90 per cent of the plants, while an equal number of controls remained free from the disease. The spots closely resembled those occurring naturally on tomato and horse nettle.

Pycnidia developed rather freely on about 1 per cent of the spots on the inoculated horse nettle plants both in the greenhouse and outdoors. The pycnidia and spores were indistinguishable from those of *Septoria lycopersici* on tomato.

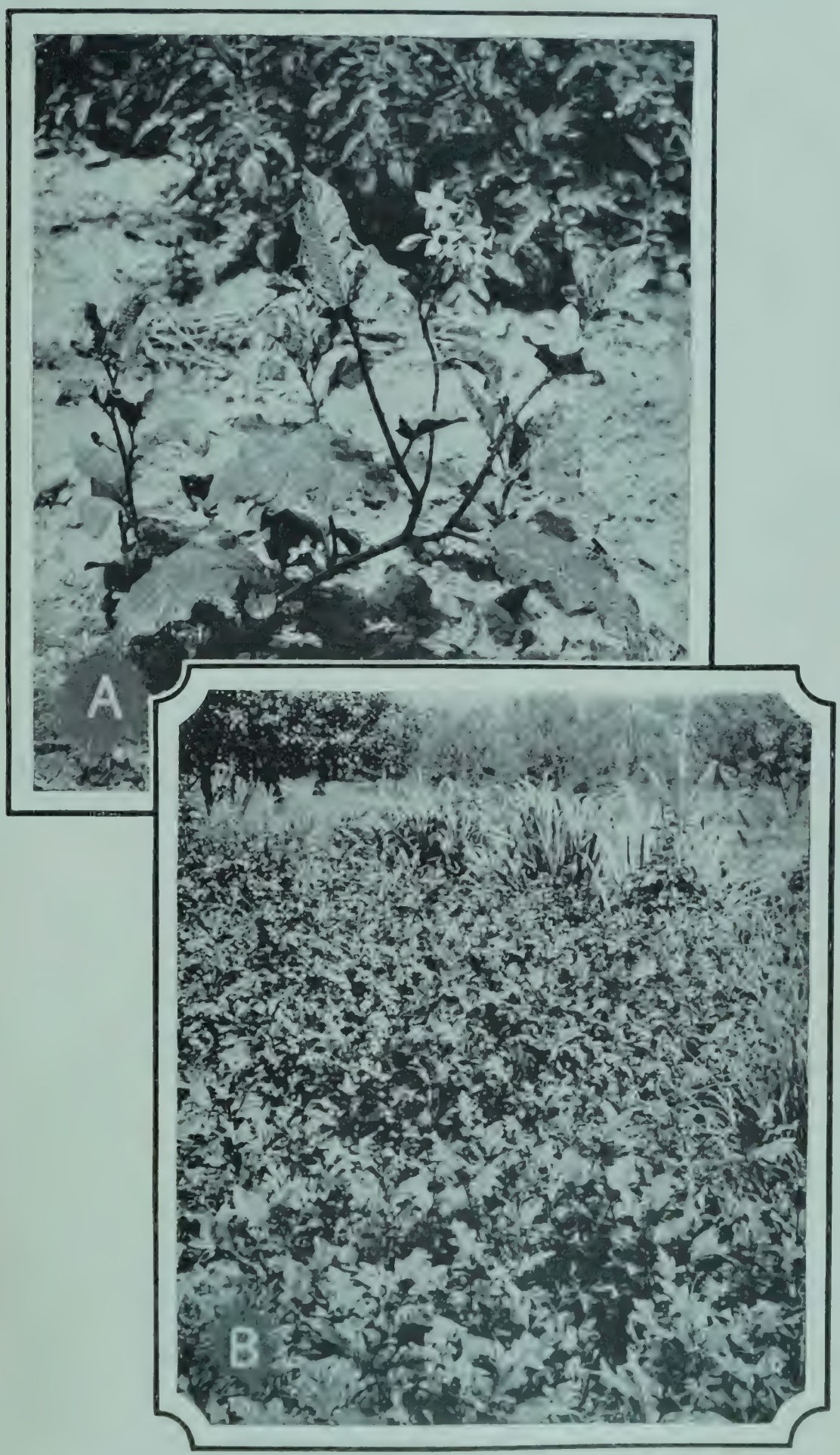
A culture of Septoria reisolated from the artificially infected horse nettle plants produced on both horse nettle and tomato leaves spots, pycnidia, and spores typical for *Septoria lycopersici*.

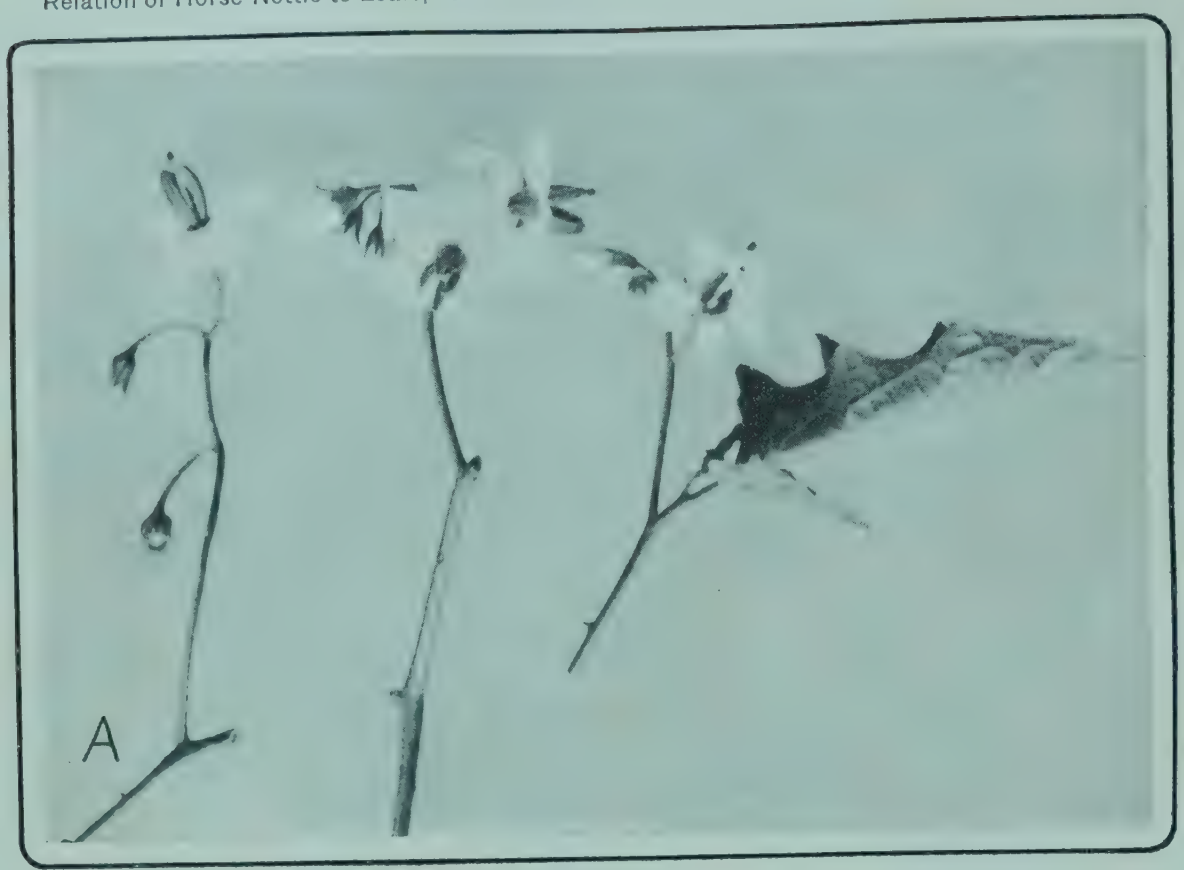
Pycnidia and spores of a Septoria identical in appearance with *Septoria lycopersici* were found on naturally infected horse nettle leaves late in the fall.

A Phoma, an Alternaria, and two species of Cladosporium were found fruiting on horse nettle leafspots, but repeated efforts to reproduce the spots with pure cultures of these fungi ended in failure. It is therefore likely that in the Middle Atlantic States most, if not all, these spots resembling leafspot of tomato are due to *Septoria lycopersici*.

PLATE 95

A.—Horse nettle plant growing in a field of tomatoes.
B.—Horse nettle plants growing on the bank of a drainage ditch.


A
B


A

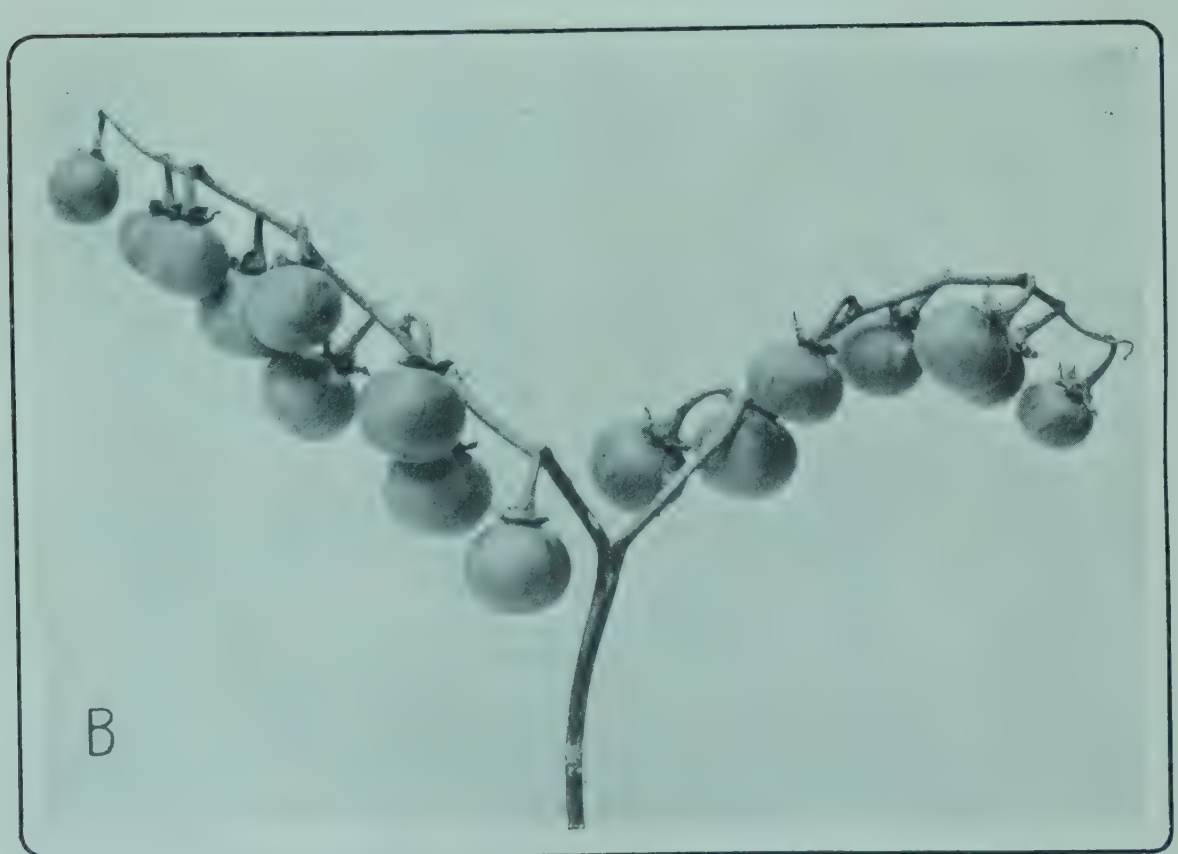

B

PLATE 96

A.—Flowers of a horse-nettle plant. Note their similarity to the flowers of potato and tomato. Some horse nettle plants have white flowers and others blue. × 2/3.

B.—Fruit, or seed balls, of a horse-nettle plant. Note their resemblance to the seed balls of potato. × 2/3.

48495°—21——7

PLATE 97

A.—Leafspot of horse nettle. Natural infection. Slightly reduced.

B.—Leafspot of horse nettle produced by inoculation with a pure culture of *Septoria lycopersici* from tomato. Enlarged to show pycnidia, which are partially embedded in the tissues of the spots. × 3.

A
B

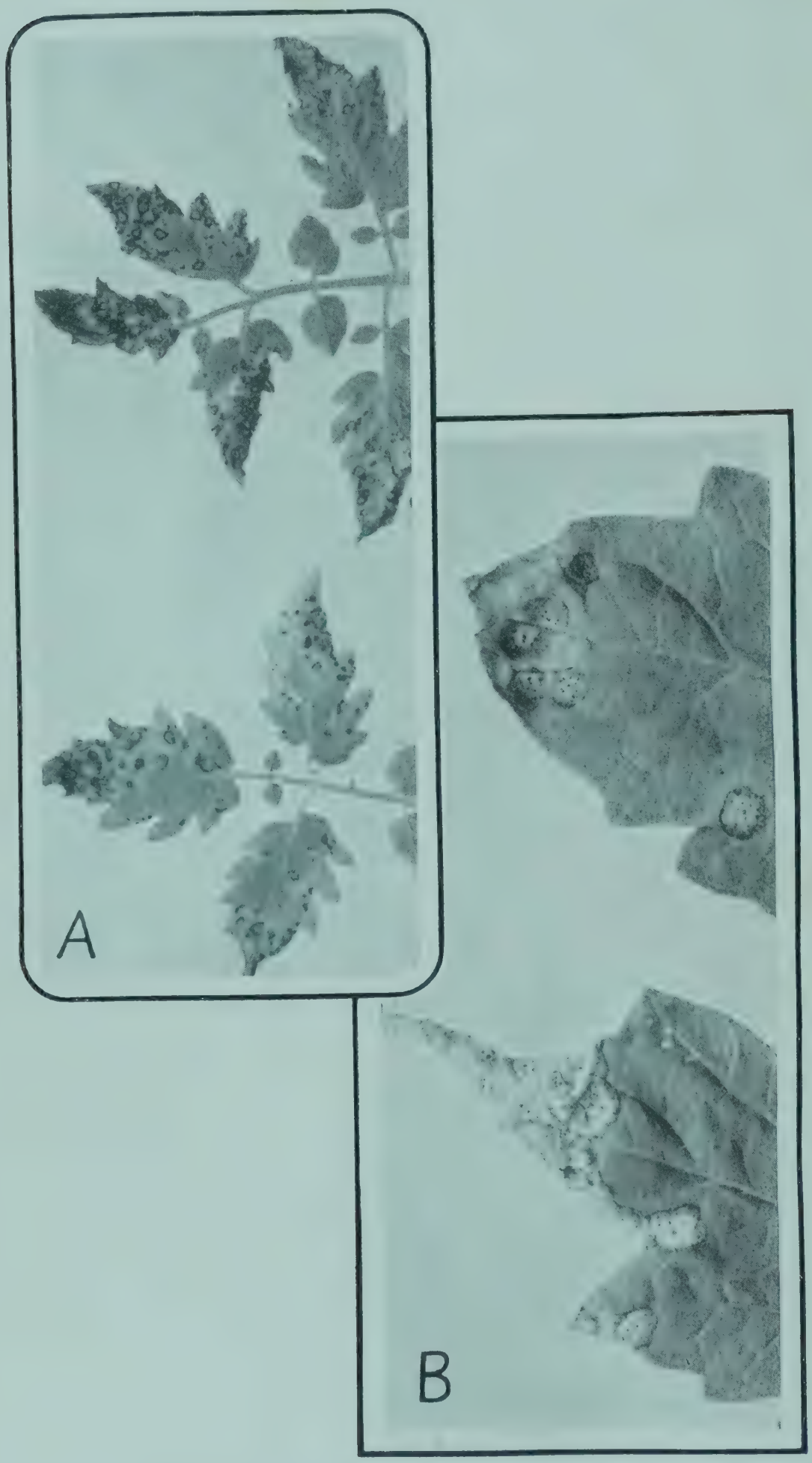

A
B

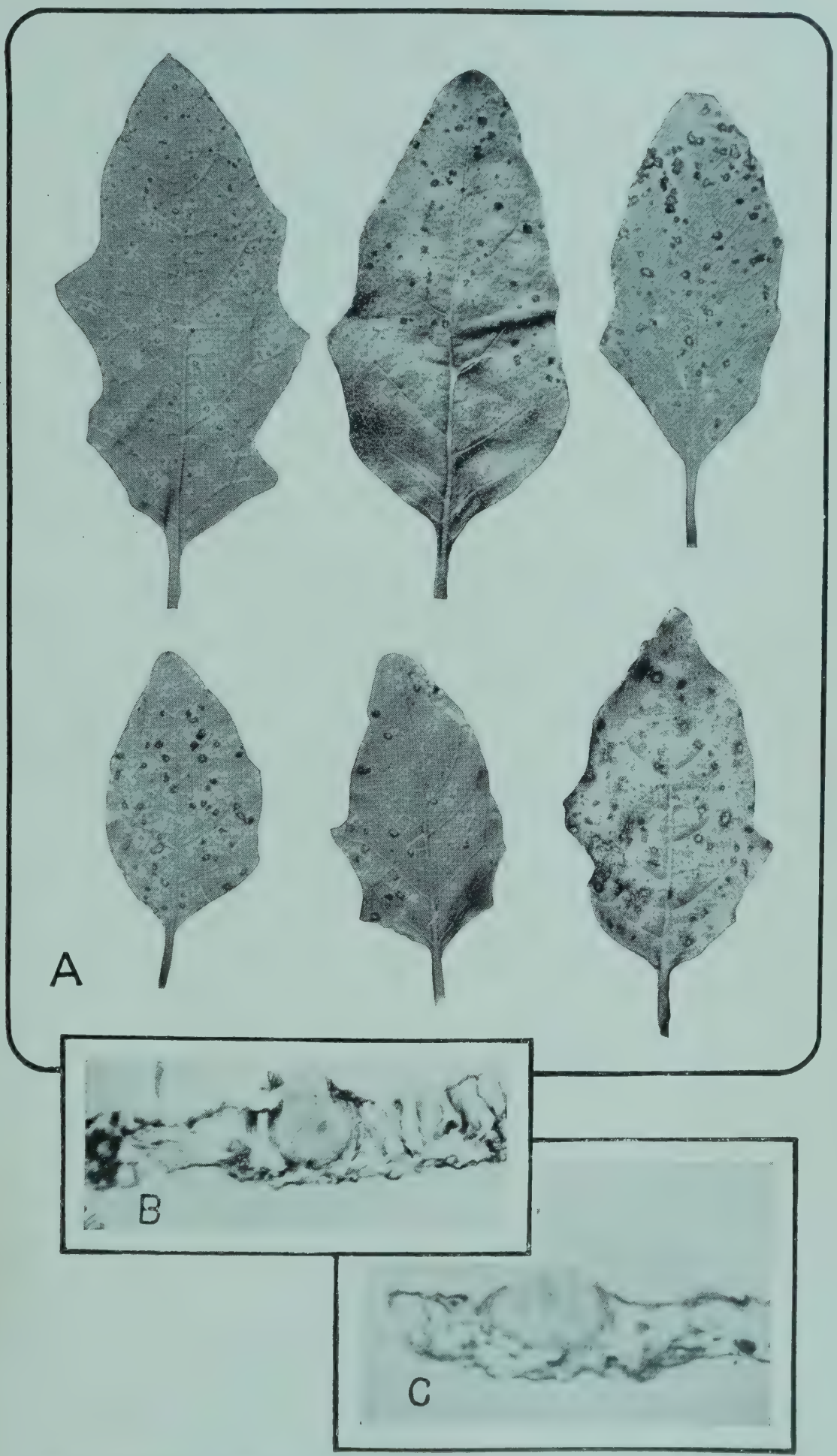
PLATE 98

Leafspot of tomato (*Septoria lycopersici*):
A.—Slightly reduced.
B.—Enlarged to show pycnidia. × 3½.

PLATE 99

A.—Leafspot of horse nettle produced by inoculation with a pure culture of *Septoria lycopersici* from a tomato leaf. Photographed two weeks after inoculation. Natural size.

B, C.—Sections of horse nettle leaf showing pycnidia and spores in mass, produced by inoculating plants with a pure culture of *S. lycopersici* from tomato.


A
B
C

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RELATION OF HARDNESS AND OTHER FACTORS TO PROTEIN CONTENT OF WHEAT

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It is well known that hard spring and hard winter wheats are higher in protein content than the soft wheats. Since the former are uniformly higher in protein content, it has been assumed that the factors for hardness and protein content are correlated.

As early as 1904 Harper and Peters (*2*)[1] made the following observation regarding wheats used in their experiments:

> The dark flinty kernels are distinctly harder than the light starchy ones and contain more protein.

Snyder (*7*) reported the analyses for protein of 63 different varieties of wheat, in which each variety was divided into two samples, one containing only light-colored (starchy) and the other only dark-colored (flinty) grains. No hardness test was made directly. The dark kernels were found to contain 15.13 per cent protein as compared with 12.76 per cent for the light-colored kernels. This is a difference of 2.37 per cent in favor of the former.

In another test (*8*) six different samples of wheat were divided into starchy and flinty kernels and analyzed for protein. The flinty kernels contained 14.04 per cent of protein and the starchy kernels 12.12 per cent. In another lot of 12 varieties the starchy kernels contained on the average 12.86 per cent protein and the flinty kernels 14.91 per cent. Averaging all of Snyder's results, there is a difference of 2.11 per cent in favor of the flinty kernels.

Snyder (*8*) also reported the analyses of starchy and flinty kernels of other grains. In all cases the flinty kernels contained the most protein, the difference being 0.82 per cent for corn, 0.75 per cent for rye, and 0.49 per cent for oats.

Shepard (*6*), of the South Dakota Agricultural Experiment Station, reported a protein content of 15.6 per cent for durum wheat and 13.68 per cent for common wheat.

[1] Reference is made by number (italic) to "Literature cited," p. 521–522.

Because of the assumed correlation between hardness and protein content, the commercial grading of wheat and the improvement of wheat by selection is based to a considerable extent on the hardness of the grain. The investigations reported in this paper had reference to improvement in the quality of wheat by selection. Since the protein content of the grain is one of the chief factors that determine quality, the question which formed the central object of this investigation was whether a method of selecting for protein content could be devised that would not involve the necessity of making chemical analyses at first, where hundreds of strains or races of wheat are being tested.

It is evident that, if hardness and protein content are genetically interdependent, this fact can be proved by means of the coefficient of correlation. It would not be possible, of course, to demonstrate the linkage of factors for any individual case. Likewise a parallel association of hardness and protein content might exist without any genetic relationship, so that a positive coefficient would not of itself prove such a relationship but would simply suggest the possibility of its presence. On the other hand, a negative coefficient would preclude such a supposition.

For the purpose of the investigation, 94 pure strains of wheat, each the product of a single head, were chosen, and the correlation coefficient between hardness and the protein content was calculated. Hardness was expressed in terms of the crushing point. The method of determining the crushing point has been described elsewhere (*3*).

For this determination 500 grains were used, it having been determined that this number gives accurate results. The analyses for protein were made by the department of chemistry of the Kansas Agricultural Experiment Station. The crushing points and percentages of protein are given in Table I

TABLE I.—*Crushing points and protein content of 94 pure strains of Kansas-grown wheat*

Serial No.	Variety.	Crushing point.	Protein content.
		Gm	*Per cent.*
909	Fuchs	3, 902	11. 57
812	Griechischer Sommer von Volo	4, 260	12. 24
895	Touzelle	5, 018	15. 52
839	Fenton	5, 313	11. 48
659	Blanc à duvet velouté	5, 407	11. 68
980	North Allerton	5, 702	10. 24
891	Duroselle	6, 009	10. 60
864	Jones Winter Fife	6, 309	12. 04
865	.....do	6, 319	10. 96
943	Bastard	6, 328	1. 36
871	Urtoba	6, 340	10. 97
1117	Turkey	6, 401	13. 20
1371	U. S. Cereal Investigation No. 1656	6, 410	10. 68
1143	Japanese Velvet Chaff	6, 494	10. 72
886	Japhet	6, 516	10. 48

TABLE I.—*Crushing points and protein content of 94 pure strains of Kansas-grown wheat*—Continued

Serial No.	Variety.	Crushing point.	Protein content.
		Gm.	*Per cent.*
781	An Australian hybrid	6,523	12.28
890	Duroselle	6,588	11.28
859	Banat	6,662	12.48
915	Paine's Defiance	6,674	11.68
852	Shirreff's Square Head	6,712	10.21
887	Japhet	6,724	10.56
1107	Kharkof	6,894	12.36
801	An Australian hybrid	6,947	12.65
802	.....do	6,950	12.32
724	Kubanka	7,086	10.24
1162	U. S. Cereal Investigation No. 1787	7,266	12.88
972	Talavera	7,496	11.36
854	Shirreff's Square Head	7,578	12.00
1159	Turkey	7,633	12.00
764	Crimean (U. S. Cereal Investigation No. 1433)	7,657	11.72
1000	Hickling	7,825	10.96
1115	Turkey	8,021	12.36
778	An Australian hybrid	8,064	13.88
694	U. S. Cereal Investigation No. 1665	8,084	12.28
1149	Japanese Square Head	8,295	11.64
842	Fenton	8,350	12.62
191	An Australian hybrid	8,504	12.26
860	Banat	8,545	12.16
1082–2	Turkey	8,719	12.12
1157	.....do	8,884	12.00
882	Japhet	9,078	12.80
977	Spalding's Prolific	9,215	11.20
973	Talavera	9,225	12.62
837	Tagenrog	9,234	11.12
761	Crimean (U. S. Cereal Investigation No. 1435)	9,242	11.36
762	.....do	9,391	11.60
893	Duroselle	9,395	10.60
1372–5	Spelt	9,484	11.84
773	An Australian hybrid	9,535	11.80
725	Kubanka	9,617	11.64
754	Beloglina	9,682	10.92
927	California March	9,756	12.08
711	U. S. Cereal Investigation No. 1654	9,825	11.08
772	An Australian hybrid	9,956	11.72
800	.....do	9,961	12.60
1116	Turkey	10,010	11.52
1081	.....do	10,016	12.52
1064	Macaroni	10,076	11.76
861	Aegyptischer	10,162	12.08
735	———	10,176	11.36
813	Griechischer Sommer von Volo	10,188	12.73
1105	Banat	10,251	11.92
1098	———	10,458	11.35
732	———	10,522	11.30
707	Red Winter	10,546	11.32
929	California March	10,592	11.65
1119	Bacska	10,594	10.94
706	Red Winter	10,645	10.89
734	———	10,765	11.36
1150	Turkey	10,776	12.00
150	Beloglina	10,899	11.67
1128	Crimean	10,855	10.84
752	Beloglina	10,972	13.04
1103	Diehl's Mediterranean	11,017	10.60
940	Bastard	11,309	11.42

TABLE I.—*Crushing points and protein content of 94 pure strains of Kansas-grown wheat*—Continued.

Serial No.	Variety.	Crushing point.	Protein content.
		Gm.	*Per cent.*
722	Ghirka	11, 323	11. 40
1074	Jones Fife×Red Winter	11, 328	11. 04
678	Red Winter	11, 438	12. 25
1152	Turkey	11, 444	11. 80
1073	Bart Gross-körniger	11, 489	11. 92
676	Red Winter	11, 554	11. 42
1082–1	Turkey	11, 567	12. 48
1110	.....do	11, 702	11. 80
774	An Australian hybrid	11, 712	11. 83
1018	Mareuil	11, 877	12. 00
1111	Turkey	11, 912	12. 00
936	Bucanera	12, 429	10. 60
1106	Banat	11, 994	11. 35
1036	Romanella	12, 442	10. 76
889	Red Summer Emmer	12, 487	11. 36
1161	U. S. Cereal Investigation No. 1787	12, 753	11. 60
1080	U. S. Cereal Investigation No. 1543	12, 873	13. 20
1066	Macaroni	12, 905	12. 76
1112	Turkey	12, 946	11. 56
	Average	9, 115	11. 73

The correlation between hardness (subject) and protein (relative) is shown in Table II. Table III summarizes the chief constants connected with the calculations.

It is apparent that the correlation between hardness and protein is practically zero. Since this is contrary to common opinion, data from

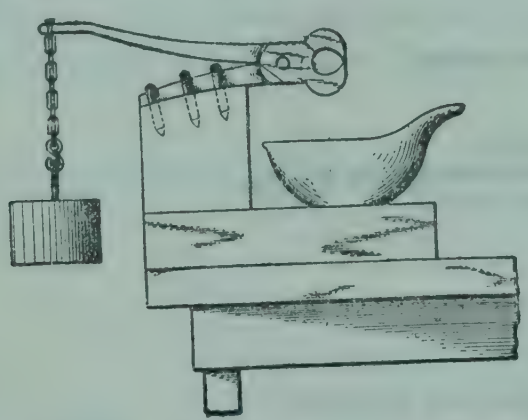

FIG. 1.—Machine used at the California Agricultural Experiment Station for determining the hardness of grain (*4*).

the California Agricultural Experiment Station and from the Kentucky Agricultural Experiment Station—the only existing data aside from those obtained in the present investigations—were examined.

The method used at the California Agricultural Experiment Station for testing the hardness of wheat is that devised and described by Shaw and Gaumnitz (*4*). The apparatus consists simply of a pair of ordinary pliers, one arm of which is stapled to a wooden block (fig. 1).

TABLE II.—*Correlation between hardness of grain (subject) and protein content (relative) in 94 pure strains of Kansas-grown wheats*[a]

			10.00 to 10.49 10.245 −1.485	10.50 to 10.99 10.745 −0.985	11.00 to 11.49 11.245 −0.485	11.50 to 11.99 11.745 −0.015	12.00 to 12.49 12.245 +0.515	12.50 to 12.99 12.745 +1.015	13.00 to 13.49 13.245 +1.515	13.50 to 13.99 13.745 +2.015	14.00 to 14.49 14.245 +2.515	14.50 to 14.99 14.745 +3.015	15.00 to 15.49 15.245 +3.515	15.50 to 15.99 15.745 +4.015	
3.000	3.499	−5.616				1									1
3.999															
4.000	4.499	−4.616					1								1
4.999															
5.000	5.499	−3.616	1		1	1								1	4
5.999															
6.000	6.499	−2.616	2	6	2	1	5	1	1						18
6.999															
7.000	7.499	−1.616	1	1	1	1	2	1							7
7.999															
8.000	8.499	− .616				1	6	1		1					9
8.999															
9.000	9.499	+ .384		2	4	5	1	3							15
9.999															
10.000	10.499	+1.384		3	5	5	2	2	1						18
10.999															
11.000	11.499	+2.384		1	5	4	4								14
11.999															
12.000	12.499	+3.384		2	1	2		1	1						7
12.999															
			4	15	19	21	21	9	3	1				1	94

[a] First three columns at left denote, respectively, classes of crushing points in kilos, mid points of classes, and deviations from the mean. The three lines in the boxes at the top denote, respectively, classes of protein percentages, mid points of classes, and deviations from the mean.

TABLE III.—*Summary of crushing points and protein content of 94 pure strains of Kansas-grown wheat*

Mean.		Standard deviation.		Correlation.
Crushing point.	Protein content.	Crushing point.	Protein content.	Coefficient (r).
Gm.	*Per cent.*	*Gm.*	*Per cent.*	
9,115 ±154.4	11.73 ±0.0015	2,219.38 ±109.17	0.8318 ±0.0409	0.0214 ±0.003

The wheat grain is placed between the jaws of the pliers, and weights sufficient to cut the kernel in two are suspended from the extremity of the upper, or fore arm, of the pliers. The writers (*4*) state:

> A number of more complicated arrangements were tried, but none seemed to give more uniform results than this simple contrivance. . . . The test was made by counting out 500 kernels, which were crushed by opening the jaws of the pliers just wide enough to insert the grain between them and then allowing the weight to settle gently. The grains remaining unbroken by each weight were set aside and counted. . . . Five different weights were employed, viz, 0.75, 1.00, 1.25, 1.50, and 1.75 pounds.

The breaking point determined in this manner is necessarily very different from the crushing point, as determined in the writer's experiment. This will be apparent by reference to the differences in the methods as described above. In order to correlate the breaking weights and protein content, the former were reduced to grams and the number of broken grains—that is, 100 less the number remaining unbroken—in each case were grouped under the separate weights. Then the sum of the products of this number—that is, the number of grains breaking under each separate weight—multiplied by the breaking weight and divided by the total number of grains crushed, gives an average breaking point in grams that rests upon a common basis with the writer's experiments, except for the differences due to the machine and the method used. The results, expressed as the mean breaking point in grams, are given in Table IV. The protein content is also given for comparison.

TABLE IV.—*Relation of mean breaking point to protein content of California-grown wheats*

WASHINGTON BLUESTEM

Laboratory No.	Number of grains broken by weight of—					Mean breaking point.	Average protein content.
	350.2 gm.	453.6 gm.	567 gm.	680.4 gm.	793.8 gm.		
						Gm.	*Per cent.*
4	0	53	80	0	0	461	8.98
206	5	23	46	0	0	517	8.85
16	0	57	72	0	0	517	10.94
5	0	57	78	0	0	519	13.71
15	0	46	83	0	0	527	10.91
9	0	17	52	0	0	539	9.05
14	0	13	69	0	0	549	12.79
10a	13	29	44	70	7	585	8.76

TABLE IV.—*Relation of mean breaking point to protein content of California-grown wheats*—Continued

WASHINGTOM BLUESTEM—continued

Laboratory No.	Number of grains broken by weight of—					Mean breaking point.	Average protien content.
	350.2 gm.	453.6 gm.	567 gm.	680.4 gm.	793.8 gm.		
						Gm.	*Per cent.*
24	7	32	59	80	0	589	11.51
25	1	46	68	92	0	591	13.87
17	0	28	52	83	0	605	11.91
386	1	23	57	88	0	609	9.53
15a	0	11	34	64	0	622	9.27
205	28	37	49	81	93	637	7.18
300	1	9	32	82	0	642	8.95
267	5	26	63	88	94	666	7.36
152	6	16	36	60	95	686	10.26
272	0	14	37	50	78	689	7.15
299	6	9	35	76	89	690	8.08
268	0	12	46	84	98	694	7.29
121	0	5	19	47	87	722	9.01
472	0	1	21	40	85	728	12.26
303	0	0	16	42	75	731	9.31
606	1	2	6	6	40	736	10.30
64	0	0	4	18	41	747	10.14
118	0	0	9	25	58	990	10.68
Average						638	9.93

AUSTRALIAN

78	0	5	42	89	0	156	11.09
309	5	8	20	37	81	271	9.83
62	0	19	53	80	0	290	11.43
288	0	4	14	44	73	294	9.63
23	61	39	11	0	0	408	12.06
141	5	50	74	0	0	514	9.90
138	8	50	77	0	0	512	10.86
134	5	46	77	0	0	518	9.90
310	0	34	82	0	0	533	8.25
184	3	20	78	0	0	538	11.07
319	0	16	57	0	0	542	8.55
66	0	0	7	25	72	560	9.86
129	0	17	50	93	0	621	9.18
127	0	9	63	96	0	625	8.27
322	0	0	7	69	0	670	8.54
275	2	21	55	87	94	676	12.56
77	0	5	42	89	0	637	10.53
452	0	1	16	23	65	688	9.43
326	4	13	28	76	86	692	10.48
232	5	14	23	72	97	697	6.92
311	0	2	9	66	0	700	8.36
69	0	5	39	78	90	702	13.36
231	0	10	12	40	57	704	7.72
67	0	2	12	39	46	715	11.41
453	0	3	15	19	53	721	10.10
22	0	1	15	54	75	725	12.52
28	0	1	15	54	75	726	12.35
26	0	1	10	16	39	727	13.42
312	0	1	8	78	88	731	7.97
73	0	0	10	39	76	740	9.83
68	0	0	10	44	84	741	11.02
605	0	0	0	10	47	773	10.83
Average						598	12.32

TABLE IV.—*Relation of mean breaking point to protein content of California-grown wheats*—Continued

LITTLE CLUB

Laboratory No.	Number of grains broken by weight of—					Mean breaking point.	Average protein content.
	350.2 gm.	453.6 gm.	568 gm.	680.4 gm.	793.8 gm.		
						Gm.	*Per cent.*
50a	77	0	0	0	0	350	8.97
51a	53	79	91	0	0	475	8.40
51a	53	79	91	0	0	475	9.21
317	5	63	82	0	0	512	8.15
11	0	57	69	0	0	516	11.35
8	0	37	61	0	0	524	7.83
277	0	40	71	0	0	526	9.05
144	1	34	79	0	0	531	8.58
49	46	54	86	92	0	554	9.19
21	6	65	73	75	0	566	13.39
305	22	34	59	80	0	569	8.61
143	2	38	70	98	0	597	9.03
315	3	30	88	98	0	599	8.18
139	5	24	66	94	0	603	9.26
290	0	23	52	75	0	606	10.39
55	4	18	59	87	0	608	8.46
130	0	20	55	90	0	615	9.08
124	2	19	45	90	0	616	9.08
128	0	16	64	93	0	617	8.18
28a	6	17	48	87	0	622	9.02
60	28	57	76	90	97	624	8.77
6	0	8	33	87	0	681	13.43
56	1	12	53	83	95	687	10.13
57	1	15	46	75	95	688	8.41
161	0	8	43	80	94	698	8.08
53	0	7	42	77	93	700	8.76
59	0	8	36	71	96	704	8.34
27	2	8	28	29	93	711	13.53
80	0	4	36	70	97	718	10.38
58	0	3	37	77	99	733	8.17
Average						601	9.38

SONORA

137	3	35	76	0	0	526	10.31
257	6	32	58	83	0	592	12.84
140	0	30	71	96	0	605	12.20
249	4	9	25	25	67	691	12.05
167	2	8	29	65	93	705	14.60
63	0	7	25	74	91	710	12.16
74	0	3	13	45	75	727	11.84
76	0	0	6	34	68	745	11.38
245	0	0	7	23	66	750	11.76
33	0	4	13	27	48	775	12.88
481	0	0	0	1	20	788	10.01
Average						692	12.00

TABLE IV.—*Relation of mean breaking point to protein content of California-grown wheats*—Continued.

PROPO

Laboratory No.	Number of grains broken by weight of—					Mean breaking point.	Average protein content.
	350.2 gm.	453.6 gm.	567 gm.	680.4 gm.	793.8 gm.		
						Gm.	*Per cent.*
45	0	24	45	60	83	631	12.00
79	0	6	48	86	0	631	11.90
47	0	8	22	66	93	713	11.90
46	0	0	11	47	73	734	11.96
Average						677	11.94

The data presented in Table IV are summarized in Table V. The constants involved in the calculation of the correlation coefficient are given in Table VI, and the correlation in Table VII.

TABLE V.—*Summary of relation of mean breaking point and protein content of California-grown wheat*

Breaking point.	Protein content.	Breaking point.	Protein content.	Breaking point.	Protein content.	Breaking point.	Protein content.
Gm.	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*
350	8.97	569	8.61	637	10.53	714	8.76
419	12.06	584	8.03	642	8.95	715	11.41
461	8.98	585	8.76	660	10.48	718	10.38
475	8.40	589	11.51	666	7.36	721	10.10
475	9.21	591	13.87	667	10.83	722	9.01
492	10.86	597	9.03	675	9.96	726	9.27
512	8.15	599	8.18	677	7.79	726	13.21
515	13.00	603	9.26	681	13.43	727	9.77
516	11.35	605	11.91	686	10.26	727	10.15
517	8.85	605	12.11	687	10.13	727	13.42
517	10.94	606	10.39	688	8.41	728	12.26
519	13.71	608	8.46	689	7.15	731	7.97
524	7.83	609	9.53	690	8.08	731	9.31
526	7.80	613	11.43	691	9.33	731	9.43
526	9.05	615	9.08	694	7.29	733	8.17
527	10.91	616	9.08	698	8.08	734	10.50
531	8.58	617	8.18	698	9.54	736	10.30
534	8.25	622	9.02	700	8.76	740	9.83
538	11.07	622	9.27	702	13.36	741	11.02
539	9.05	624	8.77	703	9.38	747	10.14
542	8.55	625	9.18	704	8.34	750	8.83
549	9.90	626	8.27	710	14.60	768	8.36
549	12.79	628	11.07	710	15.80	779	8.54
554	9.19	632	11.76	711	7.72	788	10.01
560	9.08	636	6.92	711	13.53	788	9.63
566	13.39	637	7.18	713	8.76	990	10.68

TABLE VI.—*Constants involved in the calculation of correlation coefficient of mean breaking point and protein content of California wheats*

Mean.		Standard deviation.		Correlation coefficient (r).
Crushing point.	Protein content.	Crushing point.	Protein content.	
Gm.	*Per cent.*	*Gm.*	*Per cent.*	
631	9.89	89.90	1.8058	0.0111
±5.9176	±0.1194	±4.1853	±0.0841	±0.061

The results corroborate the Kansas data, with a correlation coefficient of practically zero, and a probable error larger than the correlation coefficient.

In the Kentucky experiments, conducted by Harper and Peters (*2*), the hardness of the kernels was determined by means of a piston with a

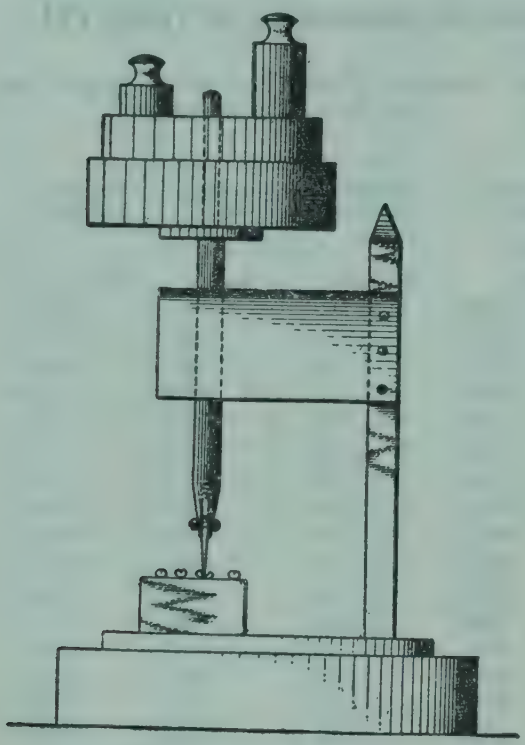

FIG. 2.—Machine used at the Kentucky Agricultural Experiment Station for testing the hardness of wheat, designed by J. N. Harper (*2*).

cutting edge, which is pressed down upon the kernel by means of weights added directly from above (fig. 2). The kernel is cut in two and is not crushed.

The opinion of the Kentucky investigators regarding the apparatus used is as follows:

> This device for testing the hardness of wheats appears to us to be superior to those that measure the pressure required to crush a kernel.

The data obtained are presented in Table VIII.

TABLE VII.—*Correlation between hardness of grain (subject) and protein content (relative) in 103 varieties of California-grown wheat* [a]

			7.00 to 7.49	7.50 to 7.99	8.00 to 8.49	8.50 to 8.99	9.00 to 9.49	9.50 to 9.99	10.00 to 10.49	10.50 to 10.99	11.00 to 11.49	11.50 to 11.99	12.00 to 12.49	12.50 to 12.99	13.00 to 13.49	13.50 to 13.99	14.00 to 14.49	14.50 to 14.99	15.00 to 15.49	15.50 to 15.99	
			7.245	7.745	8.245	8.745	9.245	9.745	10.245	10.745	11.245	11.745	12.245	12.745	13.245	13.745	14.245	14.745	15.245	15.745	
			−2.625	−2.125	−1.625	−1.125	−0.625	−0.125	+0.575	+0.875	+1.375	+1.875	+2.375	+2.875	+3.375	+3.875	+4.375	+4.875	+5.375	+5.875	
300–399	349	−290.65	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
400–499	449	−190.65	.....	.....	1	1	1	.....	.....	1	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	5
500–599	549	− 90.65	.....	2	4	5	5	1	.....	2	2	1	.....	1	2	2	.....	.....	.....	.....	27
600–699	649	+ 9.35	5	1	6	2	7	3	4	2	2	2	1	.....	1	.....	.....	.....	.....	.....	35
700–799	749	+ 10.935	.....	2	3	5	5	3	6	1	2	.....	1	.....	3	1	.....	1	.....	1	34
800–899	849	+ 20.935	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
900–999	949	+ 30.935	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
1,000–1,099	1,049	+ 40.935	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1,100–1,199	1,149	+ 50.935	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
			5	5	14	14	18	7	10	7	6	3	3	1	6	3	.....	1	.....	1	103

[a] First three columns, at left, denote, respectively, classes of breaking points in grams, mid points of classes, and deviations from the mean. The three lines in the boxes at the top denote, respectively, classes of protein percentages, mid points of classes, and deviations from the mean.

TABLE VIII.—*Protein content and hardness of Kentucky wheats*

Variety.	Protein content.	Number of kernels in 100, cut under a pressure of 4 pounds (1,818 gm.).	
		Flinty grains.	Starchy grains.
	Per cent.		
Ruby	11.63	40	56
Turkey Red	11.85	68	96
Gold Coin	12.33	94	98
Canadian hybrid	12.50	78	86
Dawson's Golden Chaff	12.63	68	92
Indiana Swamp	12.77	56	60
Harvest Queen	13.01	96	98
Hungarian	13.12	80	96
Lancaster Red	13.26	76	96
Pearl's Prolific	13.91	68	86
Fulcaster	14.01	46	68
Kansas Mortgage Lifter	14.04	56	84
Harvest King	14.06	70	90
Fultz	14.25	75	98
Jersey Fultz	14.66	80	94
Extra Early Oakley	14.68	55	66
Beechwood hybrid	14.95	76	78
Improved Rice	15.17	66	92
Pootung	16.55	50	70

The data from the Kentucky Agricultural Experiment Station are not presented in the same manner as those from the Kansas and the California Stations and can not be considered in exactly the same way. Instead of the number of kernels crushed under different weights, the percentages of kernels crushed under the same weight (4 pounds, or 1,818 gm.) are given. The kernels are divided into two classes, flinty and starchy, and the percentage number of kernels out of 100, cut with the 4-pound weight, is given for each class.

TABLE IX.—*Constants involved in the calculation of the correlation coefficient of mean breaking point and protein content of Kentucky wheat*

Mean.		Standard deviation.		Correlation coefficient.
Percentage of kernels cut.	Protein content.	Percentage of kernels cut.	Protein content.	
	Per cent.		*Per cent.*	
68.18 ±0.2256	13.65 ±0.1553	14.58 ±1.5953	1.0039 ±0.1098	0.0335 ±0.1545

Thus, it is not possible to correlate the percentages of protein with a series of different crushing or breaking points as in the other experiments. This difficulty was avoided by calculating the correlation coefficient of the percentages of flinty kernels which were cut under the given weight

with the percentage of protein. It seems that if any correlation exists, those varieties having the lowest percentage of kernels cut—the hardest varieties—should have the highest percentages of protein, and vice versa. The results as calculated by the writer are given in Table IX. The correlation table is given in Table X.

Here also the correlation between the hardness of the wheat kernels and the protein content is not significant.

TABLE X.—*Correlation between hardness of grain (subject) and protein content (relative) in 19 varieties of Kentucky-grown wheat* [a]

			11.00 to 11.99 11.495 −2.155	12.00 to 12.99 12.495 −1.155	13.00 to 13.99 13.495 −0.155	14.00 to 14.99 14.495 +0.845	15.00 to 15.99 15.495 +1.845	16.00 to 16.99 16.495 +2.845	
40 49	44.5	−23.7	1	.....	.....	1	.....	.....	2
50 59	54.5	−13.7	.....	1	.....	2	1	.....	4
60 69	64.5	− 3.7	1	1	1	.....	1	.....	4
70 79	74.5	+ 6.3	.....	1	1	3	.....	.....	5
80 89	84.5	+16.3	.....	.....	1	1	.....	.....	2
90 99	94.5	+26.3	.....	1	1	.....	.....	.....	2
			2	4	4	7	2	.....	19

[a] First three columns at left denote, respectively, classes of percentages of flinty kernels cut under pressure of 4 pounds (1,818 gm.), mid points of classes, and deviations from the mean. The three lines in the boxes at the top denote, respectively, classes of protein percentages, mid points of classes, and deviations from the mean.

TABLE XI.—*Coefficient of correlation constants*

Number of strains.	Mean.			Standard deviation.			Correlation coefficient.	
	Specific gravity.	Protein content.	Volume.	Specific gravity.	Protein content.	Volume.	Between specific gravity and protein content.	Between volume and protein content.
		Per cent.	*Mm.*		*Per cent.*	*Mm.*		
67	1.32	11.67	.....	0.04	0.754	.....	.....	.....
	±0.003	±0.062		±0.002	±0.062			
68	.....	11.67	20.60	.....	0.73	1.0089	0.0587	0
		±0.059	±0.082		±0.042	±0.057	±0.081	

The correlation coefficient between hardness and protein content proving to be practically zero, a study was then made to determine the correlation between specific gravity and protein content and between kernel volume and protein content of the Kansas pure strains. The correla-

tions are given in Tables XII and XIII. The chief constants in connection with the calculation of the correlation coefficient are presented in Table XI.

TABLE XII.—*Correlation between specific gravity (subject) and protein (relative) in 67 strains of Kansas-grown wheat* [a]

			10.00 to 10.49 10.245 1.425	10.50 to 10.99 10.745 .925	11.00 to 11.49 11.245 .425	11.50 to 11.99 11.745 .075	12.00 to 12.49 12.245 .525	12.50 to 12.99 12.745 1.075	13.00 to 13.49 13.245 1.575	
1.10 1.14	1.12	0.20	.....	.....	.....	.....	.....	.....	.....	...
1.15 1.19	1.17	.15	.....	1	.....	.....	.....	.....	.....	1
1.20 1.24	1.22	.10	.....	.....	.....	.....	.....	.....	.....	...
1.25 1.29	1.27	.05	1	.....	2	3	4	1	1	12
1.30 1.34	1.32	.00	2	10	5	6	8	4	.....	35
1.35 1.39	1.37	.05	.....	2	4	4	5	2	1	18
1.40 1.44	1.42	.10	.....	.....	1	.....	.....	.....	.....	1
			3	13	12	13	17	7	2	67

[a] First three columns at left denote, respectively, classes of specific gravity, mid points of classes, and deviations from the mean. The three lines in the boxes at the top denote, respectively, classes of protein percentages, mid points of classes, and deviations from the mean.

TABLE XIII.—*Correlation between volume of kernel (subject) and protein (relative) in 68 strains of Kansas-grown wheat* [a]

			10.00 to 10.49 10.245 −1.425	10.50 to 10.99 10.745 −.925	11.00 to 11.49 11.245 −.425	11.50 to 11.99 11.745 +.075	12.00 to 12.49 12.245 +.575	12.50 to 12.99 12.745 +1.075	13.00 to 13.49 13.245 +1.575	
15 19	17	− 3.60	1	5	3	4	8	3	1	25
20 24	22	+ 1.40	2	7	10	7	10	2	.....	38
25 29	27	+ 6.40	.....	.....	1	1	1	.....	1	4
30 34	32	+11.40	.....	.....	1	.....	.....	.....	.....	1
			3	12	15	12	19	5	2	68

[a] First three columns at left denote, respectively, classes of volumes, mid points of classes, and deviation from the mean. The three lines in the boxes at the top denote, respectively, classes of protein percentages, mid points of classes, and deviations from the mean.

There seems to be no correlation between protein content, on the one hand, and either specific gravity or volume of the grain, on the other, as was found to be true for hardness and protein content.

The absence of correlation between hardness of the grain and percentage of protein is remarkable, in view of the very general belief that the hard wheats are relatively high in protein, and the fact that this belief is supported by strong indirect evidence.

SUMMARY

(1) It has been assumed, and there is some evidence to show, that hardness of wheat is associated with and possibly dependent upon its protein content, the harder wheats being the higher in protein.

(2) In the breeding of hard winter wheat, protein is one of the most important factors next to yield to be considered. This being the case, it is desirable to discover a method of selecting for protein that will dispense with the necessity of making chemical analyses of large numbers of varieties or strains under preliminary trial.

(3) If hardness and protein content are genetically related, then when large numbers are taken the coefficient of correlation should show such relationship. This being the case, the selection of strains for hardness should involve selection for protein also.

(4) Three methods of testing wheat for hardness have been devised: That of the writer at the Kansas Agricultural Experiment Station (*3*); that of Harper and Peters, of the Kentucky Agricultural Experiment Station (*2*); and that of Shaw and Gaumnitz, of the California Agricultural Experiment Station (*4*). Calculating the correlation between the crushing or breaking point of the kernel in grams and the percentage of protein for the data from the three Stations, we have for the Kansas data a correlation coefficient of only 0.02 ± 0.003, for the California data 0.01 ± 0.061, and for the Kentucky data 0.03 ± 0.1545. These consistently negative results from the three sources seem inexplicable in the light of the generally accepted belief that the harder wheats are usually higher in protein.

(5) The correlation coefficient between specific gravity and protein and between volume of the grain and protein were also determined. The correlation coefficient was found to be $0.05 \pm$ in the first instance and zero in the second.

(6) The writer is at a loss thus far to account for the conflict between the generally recognized fact, on the one hand, that the higher protein wheats are the harder wheats, and the entire absence of demonstrable correlation between protein content and hardness by means of the correlation coefficient, on the other.

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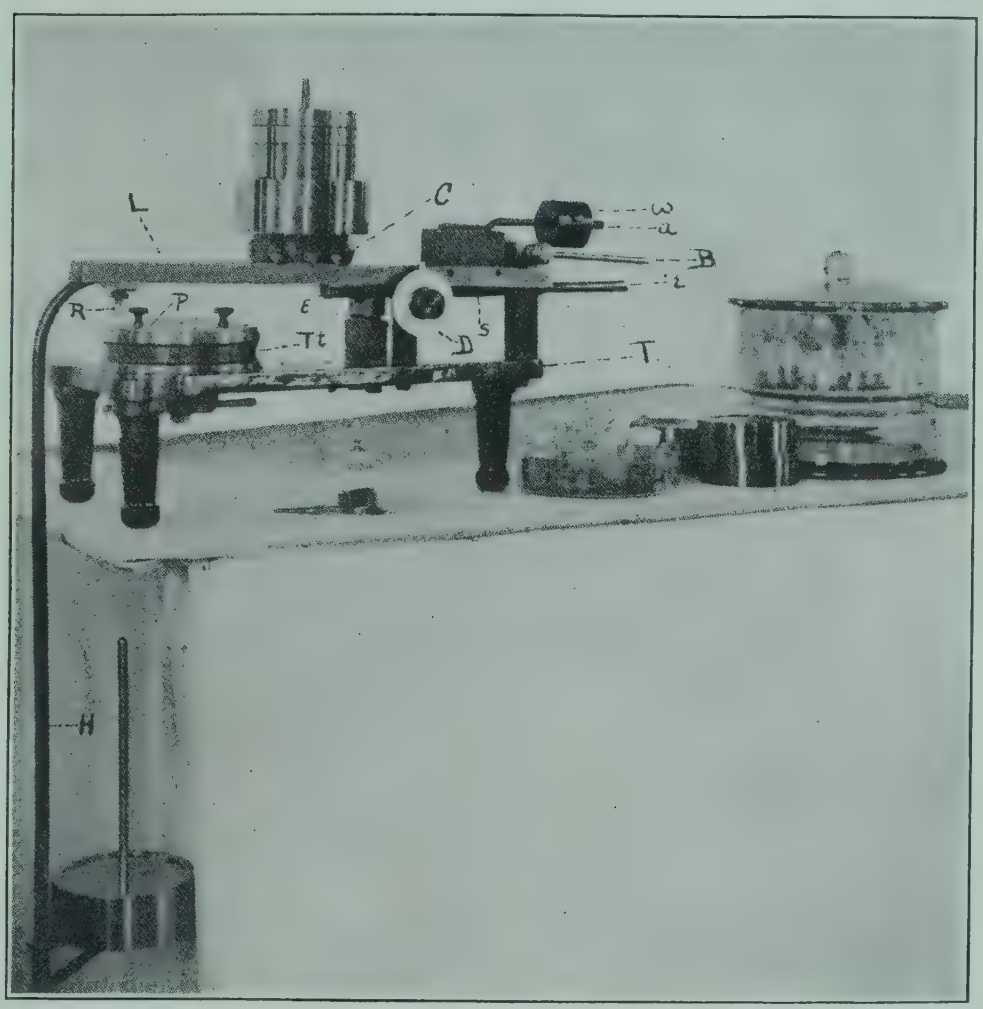
PLATE 100

Machine for determining the hardness of grain in terms of the crushing point, designed by the writer for use at the Kansas Agricultural Experiment Station (*3*). *T*, table; *L*, lever arm graduated to tenths and hundredths; *C*, carrier which runs on lever arm as a track on flanged wheels; *D*, dial which connects with sliding arm, *r*, which in turn moves the carrier *C*; *H*, hanger which rests upon lever *L* by a knife-edge bearing surface. At base of hanger is a support with upright pin upon which perforated weights are hung. *R*, ram or hammer fastened to under side of lever arm; *P*, pans in turntable upon which the grains are laid for crushing; *Tt*, turntable revolved on notched gear by means of spokes underneath; *W*, counterweight adjustable along arm *a*. Weights are brass and are in 1-, 2-, and 5-kgm. pieces, calibrated by the United States Bureau of Standards.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EFFECTS OF SOME CUCURBITA SEEDS ON ANIMAL METABOLISM

By BENJAMIN MASUROVSKY [1]

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I. INFLUENCE OF PUMPKIN AND SQUASH SEEDS UPON KIDNEY EXCRETION

The plants of the pumpkin family, *Cucurbita pepo* L., *C. moschata* Duchesne, and *C. maxima* Duchesne, are natives of the American continent, but the fruits of both pumpkin and squash are used to a greater extent in some European countries than in the United States. For instance, in Ukraine (Little Russia) the pulp is fed to the farm live stock and some seeds are given to the farm animals when the entire fruit is fed, but a large quantity of the pumpkin and squash seeds is consumed as a dainty by the people.

PURPOSE AND PLAN OF EXPERIMENT

The belief is held by many farmers that the seeds of pumpkin and squash have injurious effects upon the animal when fed in the amounts found in the entire fruit, but no careful experiments are on record to disprove the belief that unless the pumpkin and squash seeds are fed in excess they are not harmful. The purpose of this experiment is to throw some light on this question.

Since the main objection to feeding whole pumpkins lies in the opinion that the seeds cause abnormal urination, the writer of this paper experimented on himself by including in the daily diet given amounts of pumpkin and squash seeds freed from the seed coat and analyzing the daily volumes of the kidney excretion.

The experiment was carried on in two distinct periods, each period consisting of three consecutive days. The first period included March 17, 18, and 19. The second period[2] covered March 24, 25, and 26. During the first period a basal diet was followed, while during the second period an addition of given quantities of pumpkin and squash seeds without the hulls was used.

Although data of all the three days for each period are given in the tables, the results of only the last two days of each period were taken for comparison. The reason was that the results of the first day of each period might represent partial metabolic products from the day previous to the experiment.

[1] I wish to express my thanks to Dr. A. R. Moore, head of the Biological Dept. of Rutgers College, for helpful suggestions in this work and to Prof. H. R. Lewis, Poultry Husbandman at the New Jersey Agricultural Experiment Station, Prof. Frank G. Helyar, Prof. W. Ch. Skelly, and Mr. J. Thompson for assistance with the experiments.

[2] During March 20, 21, 22, and 23 small quantities of pumpkin and squash seeds were included in the diet to accustom the digestive system to the material to be used in the second period of the experiment.

BASAL DIET (PERIOD I)

The basal diet consisted of the following meals for each day: Morning, one cup of coffee, three wheat biscuits, one bowl of oatmeal and milk, and some oleomargarin; noon, two fried eggs, one glass of water, a quantity of rice pudding, three slices of bread, one cup of coffee, and some oleomargarin; evening, 1 pint of milk and 4 wheat biscuits. Since the meals were served at the same restaurant during both periods, the quantities of oatmeal, rice pudding, and bread and butter (oleomargarin) were approximately the same.

The bladder was emptied about 7 a. m. and the urine discarded. All the urine from that hour up to and including that passed the next day at 7 a. m. was saved, thoroughly mixed, preserved with toluene, and kept in 1-liter Erlenmeyer flasks with rubber stoppers. Thus, each composite sample represented the mixed excretion for 24 hours. The preservative, C. P. toluene ($C_6H_5.CH_3$), was used at the rate of 5 cc. per liter of content.

As soon as a 24-hour sample was secured it was immediately analyzed for the determination of volume, specific gravity, titratable acidity (using *N/10* sodium hydroxid), and ammonia content, employing the formol titration method of Malfatti (*3, p. 502*).[1] The total solids of a sample were computed by means of Long's coefficient (*3, p. 483*). During the intermediary period, including March 21, 22, 23, and 24, the estimation of creatinin by Folin's colorimetric method (*3, p. 506*) and the determination of total nitrogen by the Folin-Farmer microchemical method (*3, p. 485*) was accomplished for the three composite samples of the first period.

TABLE I.—*Analyses of composite samples of urine from basal diet period*

Date.	Volume.	Temperature.	Specific gravity.	Total solids.	Acidity.[a]	Ammonium hydrates (NH_3).	Creatinin.	Total nitrogen.
	Cc.	*°C.*		*Gm.*		*Gm.*	*Gm.*	*Gm.*
Mar. 17.....	1,170	25	1.024	73.008	383.70	1.02314	1.404	8.353
18.....	740	25	1.031	59.64	441.04	1.03348	1.361	6.364
19.....	650	25	1.033	55.77	451.88	0.9447	.993	7.462

[a] Expressed in cubic centimeters of *N/10* sodium hydroxid required to titrate 100 cc. of sample

EXPERIMENTAL DIET (PERIOD II)

The pumpkin and squash seeds used in the experimental diet were obtained from a canning factory where pumpkin and squash fruits are packed. To increase the palatability and to remove the effects of possible unsanitary handling of the pumpkin and squash seeds they were spread in a thin layer on a tray and placed in a dry heat oven where the material was kept at a temperature of about 150° C. until a slight browning of the seed coats was noticed, indicating that the heating had been sufficient to destroy all bacteria present and to roast the

[1] Reference is made by number (italic) to "Literature cited," pp. 358–539.

kernels. This operation also lightened the process of removing the hulls from the seeds. The weight of the seed consumed per day was taken by subtracting the weight of the hulls from the amount of seeds used.

The experimental diet consisted of practically the same meals as the basal diet, except for the addition of pumkpin and squash seeds to the evening meals. On March 24, 91 gm. of pumpkin and squash seeds were eaten;[1] on March 25, 31 gm., and on March 26, 38 gm. The meal hours and study hours during this period II were exactly the same as in period I, and all other controllable conditions were kept as uniform as possible with those of period I.

The same methods of sampling and analyzing employed in period I were used in period II.

TABLE II.—*Analyses of composite samples of urine from experimental diet period*

Date.	Volume.	Temperature.	Specific gravity.	Total solids.	Acidity.[a]	Ammonium hydrate (NH_3).	Creatinin.	Total nitrogen.
	Cc.	*° C.*		*Gm.*		*Gm.*	*Gm.*	*Gm.*
Mar. 24[b]	1, 195	25	1. 022	68. 35	415. 8	1. 10274	0. 997	9. 191
25	570	25	1. 037	54. 83	465. 1	1. 01688	. 721	4. 651
26	545	25	1. 038	55. 26	525. 7	1. 1846	. 735	7. 913

[a] Expressed in cubic centimeters of *N/10* sodium hydroxid necessary to titrate 100 cc of sample.

[b] The slightly greater volume of urine excreted during the first day (Mar. 24) of period II as compared with that of March 17 of period I is ascribed to the abnormal condition resulting from the excess of pumpkin and squash seeds ingested at the evening meal of that day.

The estimation of the total phosphoric acid (P_2O_5) and total sulphuric acid (SO_3) of the samples of both periods was done during the latter part of April. The uranium acetate method (*3, p. 552*) was employed for the determination of P_2O_5 and Folin's method (*3, p. 546*) was used for the estimation of SO_3. Table III shows the results for both periods.

TABLE III.—*Determination of P_2O_5 and SO_3*

Date of sample.	P_2O_5.	SO_3.	Mean of the last two samples.	
			P_2O_5.	SO_3.
Period I:	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Mar. 17	3. 229	1. 876	.....	.....
18	2. 434	1. 888	2. 484	1. 870
19	2. 535	1. 853		
Period II:				
Mar. 24	2. 909	1. 944	.....	.....
25	2. 513	2. 321	2. 578	2. 300
26	2. 643	2. 280		

[1] About eight hours after the evening meal on March 24 acute stomach pain accompanied by eructation into the mouth of a very acid solution took place. To relieve this condition the back of the pharynx was irritated with the finger, causing vomiting.

The content of total sulphates was almost 123 per cent in the hypothetical mean sample of period II as compared with the same of period I.

COMPOSITION OF SEEDS

The composition of the pumpkin and squash seeds (less the hulls) which were used during period II of this experiment is given in Table IV.

TABLE IV.—*Commercial analysis of pumpkin and squash seeds (less hulls)*

Ingredient.	Per cent.	Ingredient.	Per cent.
Protein	33.64	Fiber	1.42
Fat	53.48	Water	2.77

The low water content was due to the fact that the kernels were roasted. The high percentage of fat and protein keeps the same relation to the water content in raw seeds.

Comparing the results shown in Tables I and II, it is noted that not only the volume of kidney excretion during the experimental diet period is considerably less than that during the basal diet period but also the amount of total solids is about 5 per cent less in the former as compared with the total solids calculated for the excretion during the basal diet period. The marked differences are well illustrated in the supplementary Tables V and VI.

TABLE V.—*Comparison of volume of urine excreted during periods I and II*

Date of sample.	Volume.	Hypothetical mean.	Percentage of volume of period II as compared with that of of period I taken as 100.
Period I:	*Cc.*	*Cc.*	
Mar. 18	740	695	.....
19	650		
Period II:			
Mar. 25	570	557.5	80.2
26	545		

Since a slight increase or decrease in the output of urine, so far as volume is concerned, is not of great significance, the determination of the creatinin and total nitrogen, as found in the samples of periods I and II, was made. The results and comparison of both periods relating to the output of creatinin and total nitrogen is shown in Tables VII and VIII.

The data presented in these tables clearly indicate not only that the volume of urine and the amounts of total solids excreted during the experimental diet period were considerably less than during the basal

diet period but also that the percentages of total creatinin and total nitrogen in the urine during the experimental diet period was significantly less than during the basal diet period.

TABLE VI.—*Comparison of total solids of urine excreted during periods I and II*

Date of sample.	Total solids.	Hypothetical mean.	Percentage of total solids of period II compared with that of period I taken as 100.
Period I:	*Gm.*	*Gm.*	
Mar. 18	59.64	57.70	.....
19	55.77		
Period II:			
Mar. 25	54.83	55.04	95.3
26	55.26		

TABLE VII.—*Comparison of creatinin in urine of periods I and II*

Date of sample.	Creatinin.	Hypothetical mean.	Percentage of creatinin of period I compared with that of period II taken as 100.
Period I:	*Gm.*	*Gm.*	
Mar. 18	1.361	1.177	.....
19	.993		
Period II:			
Mar. 25	.721	.728	61.9
26	.735		

TABLE VIII.—*Comparison of total nitrogen in urine of periods I and II*

Date of sample.	Total nitrogen.	Hypothetical mean.	Percentage of total nitrogen of period I compared with that of period II taken as 100.
Period I:	*Gm.*	*Gm.*	
Mar. 18	6.364	6.913	.....
19	7.462		
Period II:			
Mar. 25	4.651	6.282	90.8
16	7.913		

Although the results of a single experiment of this nature do not suffice to disprove the belief prevailing among stockmen that pumpkin and squash seeds when fed to live stock exhibit a very marked diuretic

action, nevertheless the data presented above do illustrate a case which seems to reverse the general belief. The experimental diet contained a greater amount of protein and fat than the basal diet because of the addition of the pumpkin and squash seeds to the former. It would be expected that this increase of protein and fat constituents would tend to increase the proteid and nonproteid nitrogen content of the urinary excretion of the experimental period, but the results obtained show the contrary to be the case.

SUMMARY OF PART I

1. Contrary to the belief prevalent among stockmen, ingested pumpkin and squash seeds inhibited kidney secretion.

2. Not only is the volume of urine smaller when pumpkin and squash seeds are ingested but the total solids of the urine are diminished.

3. When pumpkin and squash seeds are included in the ration the acidity of the urine tends to rise.

4. An excess of pumpkin and squash seeds in a given ration causes digestive disorders.

5. The results of lower total nitrogen and creatinin in the urine when pumpkin and squash seeds are ingested is of a chemico-pharmacognosic significance.

II. EFFECT OF PUMPKIN SEEDS ON THE METABOLISM OF YOUNG PIGS

The data reported in part I of this paper prompted the investigation of the effect of pumpkin seeds on the metabolism of young pigs. The results of such investigation may lead to an efficient utilization of the by-products of the canning factories where pumpkin and squashes are packed.

In the experiments reported in the literature on feeding pumpkins to pigs (*1*, *2*) and to cattle (*4*, *5*, *6*) the only object was to determine the comparative economical feeding value of the fruits of the pumpkin family. Although it is stated in some of the scattered experimental work on feeding pumpkins to live stock that the claim made regarding the injurious effect of seeds is without foundation (*6*), no experimental data are available to support these statements.

The object of this experiment was to secure satisfactory data bearing directly on the influence of pumpkin seeds fed to young pigs.

EXPERIMENTAL WORK

On October 26, 1920, 18 pigs which were farrowed during August, 1920, were selected for this work. The age of these animals ranged between 2½ and 3 months. Pure-bred Duroc-Jerseys were used. The experiment was conducted under dry-lot conditions at the central hog barn of the New Jersey Agricultural Experiment Station. Until the animals were chosen for this experiment they were running with their

dams in one of the main hog lots where field corn was grown. They were weaned about three weeks prior to the experiment.[1] The males were castrated about two weeks previous to experimentation. The 18 pigs selected were divided into three lots, each consisting of 6 pigs, 3 barrows and 3 sows. The pens in which the pigs were confined were similar in every respect. Previous to experimental feeding the pigs received standard wheat middlings as a thin, wet mash, and whole corn, hand-fed.

On October 26 the animals were ear-tagged for identification and dipped in about a 3 per cent creolin solution against vermin. Four days were allowed for the animals to adapt themselves to the new conditions.

On October 30 their initial weights were taken (Table IX). Each self-feeder of each pen was filled with known amounts of yellow dent corn kernels, digester tankage, and standard wheat middlings. The "cannery" seeds used in this experiment were purchased from a canning factory where pumkpins and squashes were packed. The pumpkins fed to the animals were the ordinary sugar pumpkin (*Cucurbita pepo*) chopped up fine (about 1 cc. in size). On November 1 the animals were dewormed by administering internally through the mouth 32 drops of worm-seed oil to 1 fluid ounce of castor oil to each pig.

TABLE IX.—*Initial weights of pigs*

Lot No.	Tag No.	Sex.	Weight.
			Pounds.
I	626	Female	30.0
	627	Barrow	31.0
	628	Female	31.0
	629	do	28.0
	630	Barrow	25.5
	631	do	35.0
Total			180.5
II	632	Barrow	29.0
	633	Female	32.0
	634	do	39.0
	635	Barrow	27.0
	636	Female	32.5
	637	Barrow	42.5
Total			202.0
III	638	Barrow	42.0
	639	Female	26.0
	640	do	24.5
	641	Barrow	35.0
	642	Female	34.5
	643	Barrow	27.0
Total			189.0

[1] The caked condition of the udder of some of the dams was responsible for the rather late weaning time.

To analyze the pumpkin, a thoroughly mixed sample of several finely chopped pumpkins containing the seeds was taken, as well as a thoroughly mixed sample of several finely chopped pumpkins without the seeds. These samples were weighed and registered, then placed in a drying oven where they were kept for two days. The moisture content of the pumpkins without the seeds was 95.82 per cent; that of pumpkins with the seeds was 91.99 per cent; and that of the seeds was 56.60 per cent. The proportion of seed to entire fruit was obtained by weighing eight consecutive daily feedings of pumpkins before and after their seeds were removed. A list of the determinations follows.

Date.	Entire pumpkin.	Seeds.
	Pounds.	*Pounds.*
Nov. 13	13.5	1.5
14	12.5	1.2
15	13.5	1.5
16	14.0	1.2
17	12.5	1.5
18	15.0	1.3
19	16.0	2.2
20	10.0	1.0
Total	107.0	[a] 11.4

[a] 10.65 per cent of weight of entire pumpkin.

The Table X gives the composition of the feed used. The determinations for crude protein, crude fat, crude fiber, and moisture were made by Charles S. Cathcart, State chemist; the analyses for ash and nitrogen-free extract were made by the writer.

TABLE X.—*Composition of feed*

Feed.	Water.	Ash.	Crude protein.	Nitrogen-free extract.	Crude fiber.	Crude fat.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Digester tankage	10.46	17.84	56.56	5.32	1.47	8.35
Middlings	10.07	4.62	16.75	54.91	8.64	5.01
Dent corn	10.96	.83	9.44	73.12	1.23	4.42
"Cannery" seeds	6.45	3.51	29.06	9.31	16.17	35.50
Pumpkin pulp (dry)[a]	.....	8.92	8.77	64.25	15.17	2.89
Pumpkin seed (dry)[a]	.....	6.47	33.38	11.09	10.66	38.40

[a] Calculations were made on water-free basis.

The duration of the experiment was 62 days, beginning October 30 and closing December 31. The experiment was arranged and conducted in four periods.

Period I. Entire pumpkin tested against pumpkin less the seeds.

Period II. Entire pumpkin and ground "cannery" seeds tested against entire pumpkin.

Period III. Entire pumpkin and ground "cannery" seeds tested for their value.

Period IV. Ground "cannery" seeds in a dry mash tested for their value.

PERIOD I.—This period began October 30 at noon and closed November 27 at noon. The object during this period was to compare results obtained by feeding pumpkins from which the seeds have been removed with the results obtained by feeding the entire pumpkin. Lot I received during the first two weeks, besides the feed in the self-feeder ad libitum, finely chopped pumpkin containing 10.65 per cent of seeds. During the last two weeks of this period the percentage of seeds in the pumpkin was increased to 21.30 by adding loose seeds. Lot II received the feed found in the self-feeder and served as a control. Lot III received, besides the feed in the self-feeder ad libitum, chopped pumpkin from which the seeds were removed.

The results of this period are given in Tables XI to XIII.

TABLE XI.—*Feed consumed and effect on weight in period I*

Lot No.	Date.	Nutrients per 100 pounds live weight.				Gain or loss per hundredweight.
		Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.	
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I	Oct. 30 to Nov. 6	6.267	2.189	25.151	1.243	5.52
	Nov. 6 to Nov. 13	6.510	2.458	25.057	1.605	8.66
	Nov. 13 to Nov. 20	6.838	3.119	24.977	1.527	9.05
	Nov. 20 to Nov. 27	6.621	3.026	23.624	1.410	10.25
	Average per week	6.559	2.698	24.702	1.446	8.37
II	Oct. 30 to Nov. 6	6.706	2.108	26.768	1.122	5.19
	Nov. 6 to Nov. 13	5.599	1.957	26.752	1.312	5.88
	Nov. 13 to Nov. 20	5.524	1.995	28.211	1.018	9.55
	Nov. 20 to Nov. 27	5.744	1.958	26.683	.884	10.64
	Average per week	5.893	2.004	27.103	1.084	7.81
III	Oct. 30 to Nov. 6	6.608	2.063	26.374	1.279	10.31
	Nov. 6 to Nov. 13	4.617	1.391	17.250	1.035	—.23
	Nov. 13 to Nov. 20	5.407	1.748	23.118	1.193	6.25
	Nov. 20 to Nov. 27	5.659	1.710	21.262	1.239	6.67
	Average per week	5.572	1.728	22.001	1.186	7.66

Since a comparison of results obtained can be better made by figuring out the amount of nutrients required per pound of gain as well as the actual quantities of the different feeds consumed during a given period, these are given in Tables XII and XIII.

TABLE XII.—*Feed consumed during period I*

Lot No.	Date.	Corn per hundredweight.	Middlings per hundredweight.	Tankage per hundredweight.	Pumpkin per hundredweight.		Mean weekly temperature.[a]	
					Pulp.	Seeds.	Maximum.	Minimum.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*° F.*	*° F.*
I	Oct. 30 to Nov. 6	27.53	7.97	3.65	14.06	1.67	63	40
	Nov. 6 to Nov. 13	23.72	12.07	2.99	28.61	3.41	56	39
	Nov. 13 to Nov. 20	27.04	7.00	3.38	30.41	8.33	45	31
	Nov. 20 to Nov. 27	26.13	5.73	3.52	30.54	8.26	48	38
	Average per week	26.10	8.19	3.38	25.90	5.42	.....	.....
II	Oct. 30 to Nov. 6	30.34	7.92	4.45	.....	.....	63	40
	Nov. 6 to Nov. 13	28.32	10.82	1.97	.....	.....	56	39
	Nov. 13 to Nov. 20	33.42	6.66	2.22	.....	.....	45	31
	Nov. 20 to Nov. 27	32.45	5.07	3.24	.....	.....	48	38
	Average per week	31.14	7.61	2.97	.....	.....	.....	.....
III	Oct. 30 to Nov. 6	28.83	7.72	4.39	30.58	.....	63	40
	Nov. 6 to Nov. 13	17.17	6.71	3.11	31.41	.....	56	39
	Nov. 13 to Nov. 20	24.75	6.97	3.12	38.22	.....	45	31
	Nov. 20 to Nov. 27	21.49	7.91	3.84	37.10	.....	48	38
	Average per week	23.06	7.32	3.61	34.32	.....	.....	.....

[a] The temperature data were secured from the Cooperative Observers' Meteorological Records of New Jersey Agricultural Experiment Station, New Brunswick, N. J.

TABLE XIII.—*Nutrients required per pound of gain*

Lot No.	Date.	Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*
I	Oct. 30 to Nov. 6	1.135	0.392	4.556	0.225
	Nov 6 to Nov. 13	.751	.296	2.893	.185
	Nov. 13 to Nov. 20	.755	.344	2.759	.168
	Nov. 20 to Nov. 27	.645	.295	2.304	.137
	Average per week	.821	.331	3.128	.178
II	Oct. 30 to Nov. 6	1.292	.406	5.157	.216
	Nov. 6 to Nov. 13	.952	.332	4.549	.223
	Nov. 13 to Nov. 20	.578	.208	2.954	.106
	Nov. 20 to Nov. 27	.539	.184	2.507	.083
	Average per week	.840	.282	3.791	.157
III	Oct. 30 to Nov. 6	.640	.200	2.558	.124
	Nov. 6 to Nov. 13	.....	.....	.....	.....
	Nov. 13 to Nov. 20	.865	.279	3.698	.198
	Nov. 20 to Nov. 27	.848	.256	3.187	.185
	Average per week	.784	.245	3.147	.126

The feeding of the pumpkin was done regularly three times a day, by hand. Considering the young age of the experimental animals it was expected that they would consume 5 pounds of pumpkin per 100 pounds live weight per day, but the first week revealed that lot I would clean up the seeds at first and not go very readily after the pulp, while lot III would consume a little over 4 pounds per day per 100 pounds live weight. However, beginning with the second week the total weight of the entire pumpkin consumed by lot I would be equal to the total weight of pumpkin less the seeds consumed by lot III. During the first three weeks of this period considerable quantities of the whole seeds were found in the feces. Later on, however, this condition became rare. Toward the end of period I it became evident that pig 637 of lot II was gaining out of proportion as compared with the rest of the lot; on the other hand, pig 639 was a runt, being at a standstill. Pig 642 of lot III began to develop a rupture at the umbilicus. Before beginning period II these three pigs were discarded. To balance the numbers in the pens, pig 626 of lot I was transferred to pen No. 3, leaving five pigs per pen. Every effort was made to keep the lots as nearly uniform as possible.

The pigs were weighed weekly. The feeds of the self-feeders were weighed at the close of each week, and the weekly consumption of the feeding stuffs was thus obtained. For the computation of the consumption and the gain or loss during the week per 100 pounds live weight the initial weight of each lot of each week served as the basis for calculation.

During the second week of period I lot III consumed relatively less corn than each of the first two lots; hence, the gain of lot III was affected. With the exception of pig 630, lot I gave more favorable results throughout the entire period than either of the other two lots.

Period II.—This period began November 27 at noon and closed December 11 at noon. The object during this period was to compare results obtained by feeding known quantities of ground "cannery" seeds mixed with the chopped pumpkin (containing 10.65 per cent seeds) with the results obtained by feeding the chopped pumpkin (containing 10.65 per cent seeds) alone. Lot I received the mixture of pumpkin products, fed by hand, besides the feed contained in the self-feeder ad libitum. Lot II was on the self-feeder only, serving as a control. Lot III received chopped pumpkin (containing 10.65 per cent seeds) fed by hand, besides the foods of the self-feeder ad libitum.

Tables XIV to XVI show the results obtained during period II.

The feeding of the pumpkins to both lots was done regularly three times a day as during period I. Pig 630 of lot I, although he was a poor gainer and showed symptoms of indigestion at the end of period I and continued in this condition into period II, was retained to await further development of his illness. During the second week of period II, pig 633 of the control lot (lot II) began to scour and vomit and lost 11 pounds of her weight. The cause of sickness of pig 630, therefore,

could not well be ascribed to the presence of the pumpkins or ground seeds, for pig 633, which received no pumpkin whatsoever and was in better condition, followed the example of pig 630. For some reason unexplained, except possibly by the rainy weather during the last week of period II, none of the lots made progress as expected. At the end of period II the sick pigs, 630 and 633, were discarded. To balance up the lots, pig 640 of lot III was also expelled from the experiment, because of slow development. The remaining 12 animals were then divided into two lots. Lot I, retaining the 4 of the original animals, received pigs 641 and 643 from lot III. Lot II received pigs 626 and 628 from lot III, as an addition to its remaining 4.

TABLE XIV.—*Feed consumed and effect on weight in period II*

Lot No.	Date.	Nutrients per 100 pounds live weight.				Gain or loss per hundredweight.
		Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.	
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I	Nov. 27 to Dec. 4	6.177	3.105	22.750	1.701	13.05
	Dec. 4 to Dec. 11	5.698	3.291	18.164	1.748	−1.27
	Average per week.	5.937	3.198	20.457	1.724	.....
II	Nov. 27 to Dec. 4	5.170	1.751	23.513	1.016	12.17
	Dec. 4 to Dec. 11	4.549	1.547	20.882	.888	−4.49
	Average per week.	4.859	1.649	22.197	.952	.....
III	Nov. 27 to Dec. 4	5.484	2.160	23.701	1.270	12.75
	Dec. 4 to Dec. 11	4.844	1.968	20.639	1.142	−1.07
	Average per week.	5.164	2.064	22.170	1.206	.....

TABLE XV.—*Feed consumed during period II*

Lot No.	Date.	Corn per hundredweight.	Middlings per hundredweight.	Tankage per hundredweight.	Pumpkin per hundredweight.		"Cannery" seeds per hundredweight.
					Seeds.	Pulp.	
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I	Nov. 27 to Dec. 4	23.71	7.66	2.39	3.58	25.39	2.39
	Dec. 4 to Dec. 11	18.43	6.35	2.11	3.00	20.94	4.23
	Average per week.	21.07	7.00	2.25	3.29	23.16	3.31
II	Nov. 27 to Dec. 4	26.07	7.87	2.46	.....	.....	.....
	Dec. 4 to Dec. 11	23.46	6.58	2.18	.....	.....	.....
	Average per week.	24.76	7.22	2.32	.....	.....	.....
III	Nov. 27 to Dec. 4	25.50	7.62	2.38	1.85	23.88	.....
	Dec. 4 to Dec. 11	22.19	6.34	2.11	3.03	25.51	.....
	Average per week.	23.84	6.98	2.24	2.44	24.69	.....

TABLE XVI.—*Nutrients required per pound of gain*

Lot No.	Date.	Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.	Mean weekly temperature.	
						Maximum.	Minimum.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	°F.	°F.
I	Nov. 27 to Dec. 4	0.473	0.237	1.742	0.129	47	35
	Dec. 4 to Dec. 11	.....	.....	.....	.....	44	34
II	Nov. 27 to Dec. 4	.424	.143	1.932	.083	47	35
	Dec. 4 to Dec. 11	.....	.....	.....	.....	44	34
III	Nov. 27 to Dec. 4	.430	.169	1.858	.099	47	35
	Dec. 4 to Dec. 11	.....	.....	.....	.....	44	34

PERIOD III.—This period began December 11 at noon and closed December 24 at noon. Only two lots, I and II, were under experimentation. The object during this period was to find the approximate value of the entire pumpkin as a succulent and the ground "cannery" seeds as a concentrate in the ration for young growing pigs. During this period lot I received chopped pumpkin (containing 10.65 per cent seeds), fed by hand, and a known amount of ground "cannery" seeds mixed with the middlings of the self-feeder ad libitum. Lot II was on the self-feeder only, thus serving as a control.

Tables XVII to XIX show the results obtained during period III.

TABLE XVII.—*Feed consumed and effect on weight in period III*

Lot No.	Date.	Nutrients per 100 pounds live weight.				Gain per hundredweight.
		Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.	
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I	Dec. 11 to Dec. 18	5.802	2.812	22.311	1.267	10.81
	Dec. 18 to Dec. 24	5.435	2.514	19.343	1.082	8.32
	Average	5.618	2.663	20.827	1.174	9.56
II	Dec. 11 to Dec. 18	5.657	1.839	23.958	1.044	11.34
	Dec. 18 to Dec. 24	4.776	1.446	17.583	.940	2.02
	Average	5.216	1.642	20.720	.992	6.68

During the first week of period III no marked difference in gain and consumption of food per 100 pounds live weight took place. However, during the second week of this period lot I gained four times more weight per hundredweight, consuming a little more of each ingredient.

PERIOD IV.—This period is practically a continuation of the preceding period except for the elimination of pumpkins from the ration. The ground "cannery" seeds were mixed with the middlings and placed in the self-feeder from which the pigs could get it ad libitum. The object

during this period was to find the value of these Cucurbita seeds as a constituent of the dry mash and the possible effects on young pigs when fed ad libitum.

The results of this study are given in Tables XX to XXII.

TABLE XVIII.—*Feed consumed during period III*

Lot No.	Date.	Corn per hundredweight.	Middlings per hundredweight.	Tankage per hundredweight.	Pumpkins per hudredweight.		Cannery seeds per hundredweight.
					Seeds.	Pulp.	
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I.....	Dec. 11 to Dec. 18..	26.48	3.19	2.93	3.14	26.37	1.92
	Dec. 18 to Dec. 24..	23.23	2.26	3.32	3.18	26.70	1.66
	Average.....	24.85	2.72	3.12	3.16	26.53	1.79
II.....	Dec. 11 to Dec. 18..	26.71	7.75	3.25	.....	.....	.....
	Dec. 18 to Dec. 24..	17.97	7.79	3.14	.....	.....	.....
	Average.....	22.34	7.77	3.19	.....	.....	.....

TABLE XIX.—*Nutrients required per pound of gain*

Lot No.	Date.	Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.	Mean weekly temperature.	
						Maximum.	Minimum.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*°F.*	*°F.*
I.....	Dec. 11 to Dec. 18..	0.536	0.260	2.063	0.117	48	31
	Dec. 18 to Dec. 24..	.653	.302	2.326	.130	40	26
	Average.....	.594	.281	2.194	.123	.....	.....
II.....	Dec. 11 to Dec. 18..	.498	.162	2.112	.092	48	31
	Dec. 18 to Dec. 24..	2.364	.715	8.704	.465	40	26
	Average.....	1.431	.438	5.408	.278	.....	.....

TABLE XX.—*Feed consumed and effect on weight in period IV*

Lot No.	Date.	Nutrients per 100 pounds live weight.				Gain per hundredweight.
		Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.	
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I.....	Dec. 24 to Dec. 31.....	5.905	3.164	18.074	1.480	6.14
II.....	Dec. 24 to Dec. 31.....	5.260	1.650	20.424	1.002	8.51

The difference in gain of lot I was attributed to the abrupt change in feeding (excluding the pumpkins which were fed to them continuously

for eight weeks). No ill effects were noticed in lot I. Pig 626 of lot II showed signs of indigestion (loss of appetite). At the close of the experiment the general appearance of the animals of pen No. 1 was more favorable than that of the animals of the control lot (pen No. 1). The original four of lot I made a total gain of 148 pounds throughout the entire experiment against a total gain of 99.75 pounds made by the four original animals of the control lot, leaving a margin of 48.25 pounds in favor of lot I.

The record of each pig is given in Table XXIII.

TABLE XXI.—*Food consumed during period IV*

Lot No.	Date.	Corn per hundredweight.	Middlings per hundredweight.	Tankage per hundredweight.	"Cannery" seeds per hundredweight.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*
I.....	Dec. 24 to Dec. 31.....	20.53	4.39	3.35	4.95
II.....	Dec. 24 to Dec. 31.....	21.73	7.92	3.52	.....

TABLE XXII.—*Nutrients required per pound of gain*

Lot No.	Date.	Crude protein.	Crude fat.	Crude nitrogen-free extract.	Crude fiber.	Mean weekly temperature.	
						Maximum.	Minimum.
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*° F.*	*° F.*
I.....	Dec. 24 to Dec. 31..	0.961	0.515	2.943	0.241	38	24
II.....	Dec. 24 to Dec. 31..	.618	.193	2.400	.117	38	24

TABLE XXIII.—*Individual live weights of the pigs*

Lot No.	Tag No.	Sex.	Oct. 30.	Nov. 6.	Nov. 13.	Nov. 20.	Nov. 27.
			Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I.....	626	Female.....	30	32	36	38.5	42
	627	Barrow.....	31	32	35	39	45
	628	Female.....	31	32	37	41	44.5
	629	.....do.....	28	31.5	33	36.25	40.5
	630	Barrow.....	25.5	27.5	29	30.5	30.25
	631	.....do.....	35	35.5	37	41.5	48.5
II.....	632	.....do.....	29	30	32	36	38.5
	633	Female.....	32	34	38	41	45.25
	634	.....do.....	39	38	40	41.5	48
	635	Barrow.....	27	28.5	30	31.5	34.25
	636	Female.....	32.5	34.5	34	35.5	37.25
	637	Barrow.....	42.5	47.5	51	61	69.5
III.....	638	.....do.....	42	47	49	56	61.25
	639	Female.....	26	26.5	25	25	24.5
	640	.....do.....	24.5	24.5	25	26	28
	641	Barrow.....	35	40.5	39	37.5	37.5
	642	Female.....	34.5	38.5	37	41	43.5
	643	Barrow.....	27	31.5	33	35.5	41

TABLE XXIII.—*Individual live weights of the pigs*—Continued

Lot No.	Tag No.	Sex.	Dec. 4.	Dec. 11.	Dec. 18.	Dec. 24.	Dec. 31.
			Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
I	626	Female	48.5	46.75	53	53.5	51.75
	627	Barrow	51	51	59.5	64	71
	628	Female	50	49.75	52	56.5	58
	629	do	51	53	58	63.5	67.5
	630	Barrow	28	21.25	20	.....	.....
	631	do	56	58	62	68	76.5
II	632	do	44.5	48.75	51.5	54.25	62.75
	633	Female	46.5	35.5	31.75	.....	.....
	634	do	55	55	62.5	64.5	71.5
	635	Barrow	41	41.5	45.75	47.5	52.5
	636	Female	41	37	39.5	38.25	40.5
	637	Barrow	.....	.....	.....	.....	.....
III	638	do	67.5	70.75	81.5	82.5	90.5
	639	Female	.....	.....	.....	.....	.....
	640	do	31.5	30	35.25	.....	.....
	641	Barrow	40.5	38.75	44	45.5	45
	642	Female	.....	.....	.....	.....	.....
	643	Barrow	48.5	47.75	55	60.5	62

It is of interest to note that the content of lecithin, a growth-promoting substance, is nearly twice as great in shelled pumpkin seeds as in linseed cake (7).

SUMMARY OF PART II

1. When pumpkins are fed to very young pigs, for some reason the seeds are more readily consumed than the pulp.

2. Whole seeds appeared in feces at first.

3. Although sickness occurred, it could not be attributed to the presence of Cucurbita seeds in the ration.

4. Pumpkins, as a succulent, tend to increase the appetite.

5. Ground "cannery" seeds fed ad libitum have no detrimental effect on the metabolism of young growing pigs.

Because of individuality and other variable factors existing in lot feeding it is not wise to conclude that the feeding of the pumpkin seeds was responsible for any better results obtained, so far as gain, thrift, and general appearance of the animals is concerned. However, it is safe to say that no injurious effects have been noticed on the animals to which the pumpkin seeds were fed, whether in the fruit or when received as a refuse from the canning factory. In the opinion of the writer a period of 62 days with the very young pigs used in this work is sufficient to warrant the conclusion that the existing belief among farmers that pumpkin seeds are injurious to young pigs is groundless.

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A CONSTANT-TEMPERATURE BATH FOR HEATING BLOOD SERUM

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For heating clear anti-hog-cholera serum to a temperature of 58° to 60° C., as recommended by Dorset and Henley,[1] some form of constant-temperature bath is very desirable. Temperatures much higher than 60° are very injurious to serum, and on that account any form of heating bath should have some arrangement to prevent temperatures higher than 60°; indeed, this is of almost fundamental importance in any automatically controlled bath. When the heating bath is kept under constant personal supervision the temperature is easily regulated and kept within the desired range, but with an automatic heater there is supposed to be no necessity for close personal supervision, and a derangement of the temperature-controlling apparatus, if unnoticed, may result in the complete loss of a batch of serum unless some way of preventing temperatures injurious to the serum has been provided.

After considerable experimentation a bath has been devised which seems to embody a number of advantages. The operation of this bath depends upon the utilization of the vapors of a liquid having a boiling point at or about 61° C. as the heating medium, and the bath is so constructed that a very small quantity of the liquid may be used.

OPERATION AND DESCRIPTION OF THE BATH

A diagram of the bath as used in the laboratory is shown in figure 1. In brief, the bath consists of (1) a stand, *A;* (2) a jacketed kettle, *B*, provided with a draw-off valve, *a*, for the inner kettle, an inlet, *b*, and an outlet, *c*, to the outer jacket; (3) a cover, *C;* (4) a stirrer, *D;* (5) a condenser, *E;* (6) a liter flask, *F;* and (7) a thermometer, *T*. A perforated cork stopper, *s*, covered with tin foil, should be used to connect the flask, *F*, to the inlet of the kettle.

In operating, the draw-off valve, *a*, is closed, the inner kettle, *B*, is filled with serum, the cover, *C*, and the stirrer, *D*, are adjusted and started, the condenser, *E*, is attached, and water is allowed to flow through it. About 300 cc. of chloroform (U. S. P., boiling point 61° C.) is placed in the flask, *F*, and the flask is then attached to the inlet, *b*, of the jacket. Heat, preferably a gas flame, or any other source of plentiful, controllable

[1] Dorset, M., and Henley, R. R. Production of clear and sterilized anti-hog-cholera serum [Preliminary paper.] *In* Jour. Agr. Research, v. 6, no. 9, p. 333-338. 1916.

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heat, is applied to the flask, *F*. Heat should be supplied in abundance in the beginning so as to keep the chloroform boiling constantly and should be reduced only when the condenser capacity is exceeded. As a usual thing there is but little condensation of the chloroform in the condenser until the serum temperature exceeds 52°. In other words, until the temperature of the serum reaches 52° the kettle itself acts as a condenser.

The kettle used in the tests has a capacity of 10 liters. It has been possible to raise the temperature of this quantity of water in one hour from 15° C. to 58°, and a temperature of 58° to 60° has been maintained for six hours with a loss of only 20 cc. of chloroform. With a condenser functioning properly no loss of chloroform should occur.

LIQUIDS SUITABLE FOR HEATING MEDIUM

Of the liquids used, or considered, as heating mediums, chloroform is the most satisfactory, since (1) it has a suitable boiling point, (2) it is noninflammable, (3) it has a characteristic odor, (4) it is not poisonous, (5) it is not corrosive, (6) it is insoluble in water, and (7) it is reasonably cheap and easily obtained. Although the boiling point of chloroform is 61° C., it has never been possible to raise the temperature of the serum in the kettle above 60° even when sufficient heat was applied to overtax the condenser.

In altitudes greater than that of Washington, D. C., it may be necessary to use a liquid of higher sea-level boiling point than chloroform. It is believed that the most suitable liquid for this purpose is a mixture of carbon tetrachlorid and chloroform. Carbon tetrachlorid boils at 74° C. at sea level, and so mixtures of it and chloroform may be prepared with boiling points at sea level varying from a little above 61° to a little below 74°, depending upon the quantity of each liquid present. For example, at an altitude of 5,000 feet the boiling point of chloroform is considerably lower than 61°. In this case a mixture of carbon tetrachlorid and chloroform having a boiling point of 61° should be prepared. The proportion of carbon tetrachlorid to chloroform that will give a mixture having a boiling point of 61° for a given altitude can be easily determined by preparing several mixtures of the two liquids in varying proportions and determining the boiling point of each. In this way the proportion of carbon tetrachlorid to chloroform that is required to give a mixture with boiling point of 61° at the altitude in question may be ascertained.

If a liquid of lower boiling point than 61° C. at sea level should be desired, acetone, which has a boiling point of 58°, is suggested. Acetone, however, is inflammable and is soluble in water.

In all cases where mixtures are used, the utmost precaution should be taken to insure the perfect condensation of the vapors, since otherwise by

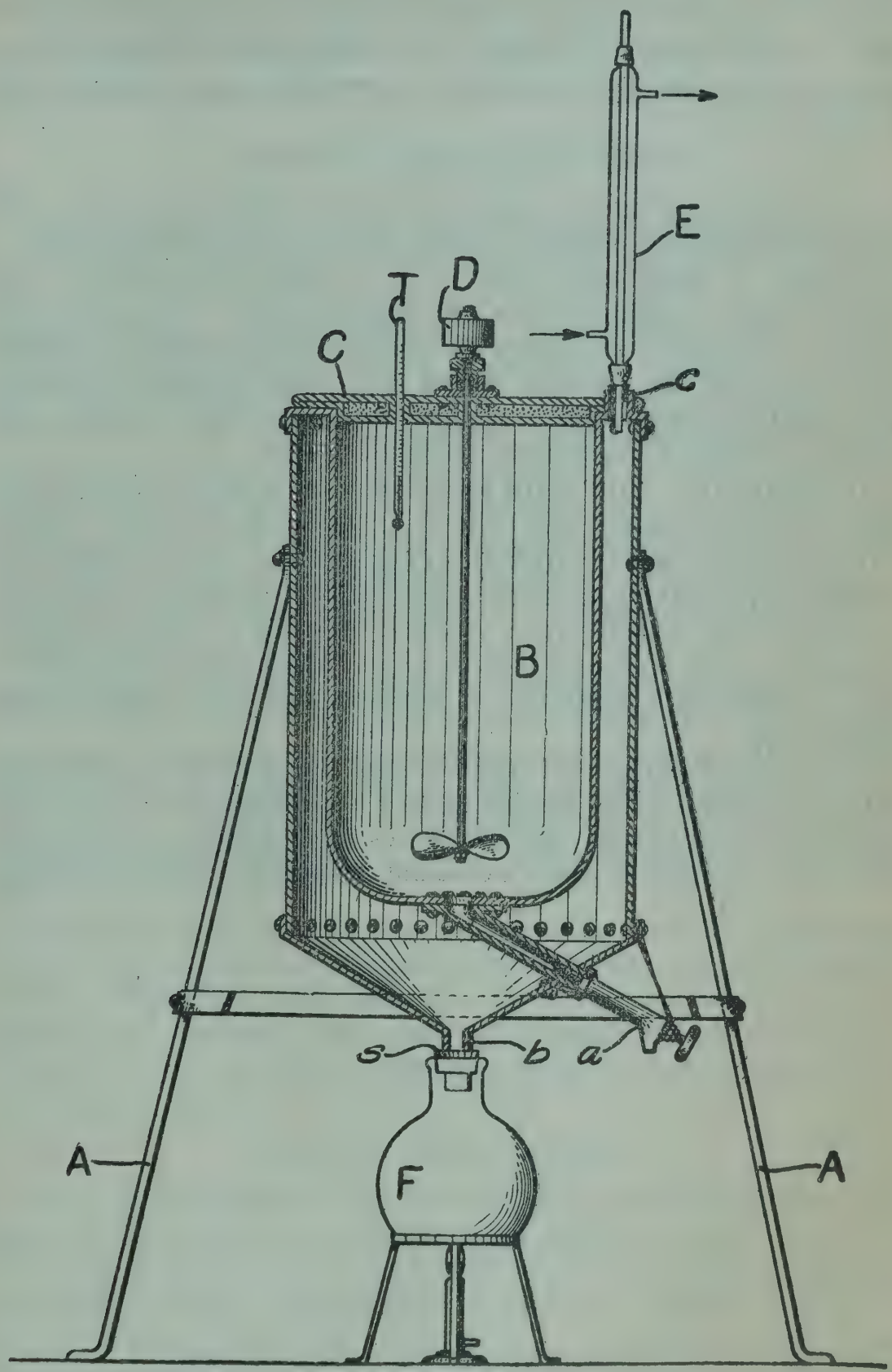

FIG. 1.—Automatic heating bath for anti-hog-cholera serum.

the escape of the more volatile constituent the boiling point of the residue is increased. The boiling point of the liquid used as a heating medium should be frequently tested, particularly when mixtures are used, in order to guard against any change. For this purpose it is advisable to have some provision made by which a thermometer may be inserted into the flask, *F*.

SPECIFICATIONS FOR APPARATUS

The kettle described was constructed as a laboratory model. For practical purposes the following general specifications are suggested:

(1) Size. A kettle larger than 10 by 20 inches should not be constructed until its feasibility has been demonstrated. The smaller the diameter of the kettle the greater is the heat-absorbing surface obtained. On this account the diameter should not exceed one-half the height. With small diameters there is a rapid flow of convection currents, and it may be possible to abolish the mechanical stirrer.

(2) Construction. The kettle should be constructed of a noncorrosive metal, without crevices, seams, or corners that can not be readily cleaned.

The advantages derived from this kettle are: (1) It is of simple construction, (2) is entirely automatic, (3) there is no danger of overheating, (4) any desired temperature is obtained by using a liquid of suitable boiling point, and (5) a minimum of a fluid, which may be expensive, is used to maintain a maximum of another fluid at a predetermined temperature.

With these advantages it is believed that the usefulness of this kettle will not be limited to the heating of clear hog-cholera serum but that it can be used for heating various serums and vaccines, or it may be used for any purpose in which a constant-temperature bath is necessary.

ASSIMILATION OF NITROGEN, PHOSPHORUS, AND POTASSIUM BY CORN WHEN NUTRIENT SALTS ARE CONFINED TO DIFFERENT ROOTS

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INTRODUCTION

In a former number of the Journal of Agricultural Research,[1] data were presented which showed that when the supply of an essential element is restricted to a portion of a plant's roots the amount of this element assimilated is diminished. The relation between the fraction of the roots supplied and the amount of the element assimilated seemed to agree with Mitscherlich's formulation of the law of minimum. Practically the same factor was obtained for the assimilation of nitrogen as for the assimilation of phosphorus, potassium, or iron. These data, however, were all obtained for one general condition—namely, where part of the plant's roots were in a complete nutrient solution and the remainder were in a nutrient solution lacking only one essential element. No tests were conducted with part of the roots in a solution lacking two or more elements.

The present paper reports work which shows how the assimilations of nitrogen, phosphorus, and potassium were affected when half the roots of the plant were in a complete nutrient solution and half in a solution lacking more than one essential element; when the roots were divided between two solutions, each of which lacked one or two elements; and when the roots were divided among three solutions, each of which lacked one or two elements.

Only nitrogen, phosphorus, and potassium were varied in these tests. It did not seem feasible to attempt to determine how the assimilations of calcium, magnesium, and sodium would be affected by localizing the supply, since relatively small amounts of these elements are absorbed by corn. Moreover, nutrient solutions lacking in these elements would probably be unfavorable for root growth.

METHOD OF EXPERIMENTS

The methods followed in conducting these experiments were very similar to those employed in the tests previously reported.

Corn (*Zea mays* L.), was used in all the following experiments because of the facility with which its roots could be divided among the

[1] GILE, P. L., and CARRERO, J. O. ABSORPTION OF NUTRIENTS AS AFFECTED BY THE NUMBER OF ROOTS SUPPLIED WITH THE NUTRIENT. *In* Jour. Agr. Research, v. 9, no. 3, p. 73-95, 2 fig. 1917. Literature cited, p. 94-95.

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different flasks. The seeds were germinated in sphagnum moss or in thoroughly leached coconut fiber. When the plumules were about 1½ inches long the seedlings were transferred to the nutrient solutions.

Two or three Erlenmeyer flasks joined together at the necks and covered with black cloth were used as containers for the nutrient solutions. One seedling was grown in each of these double or triple flasks for a period of 20 days. The plants were started in 200-cc. flasks, but as they became larger they were transferred successively to 500-cc. and 1,000-cc. flasks. By guiding the new roots into the proper flasks, the roots of each plant were kept equally divided between the two or three nutrient solutions afforded the plant.

The nutrient solutions were renewed six times during the 20-day period of each experiment, and transpired water was replaced daily. While the plants were small the solutions were changed every 4 days; later the solutions were changed every 3 days, and finally every 2 days. The frequent renewals of the solutions and the large size of the flasks insured an ample supply of the nutrients.

Rain water, caught on the roof of the glasshouse, was used in making up the nutrient solutions. It contained only 17 parts per million of total solids (organic and inorganic) and was, therefore, sufficiently pure for these experiments. In these experiments it was necessary only to guard against appreciable contamination with nitrogen, phosphoric acid, and potash. The compositions of the nutrient solutions used in the different tests are given in Table I.

TABLE I.—*Composition of nutrient solutions used*

Chemical.	Complete solution.	Solution lacking nitrogen.	Solution lacking phosphorus.	Solution lacking potassium.	Solution lacking nitrogen and phosphorus.	Solution lacking nitrogen and potassium.	Solution lacking phosphorus and potassium.	Solution lacking nitrogen, phosphorus, and potassium.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Monopotassium phosphate (KH_2PO_4)	7.14	7.14	.....	.....	.....	.....	.....	.....
Monosodium phosphate (NaH_2PO_4)	.....	.....	.....	10.06	.....	10.05	.....	.....
Potassium nitrate (KNO_3)	14.40	.....	14.40	.....	.....	.....	.....	.....
Sodium nitrate ($NaNO_3$)	9.18	.....	9.18	15.20	.....	.....	.....	.....
Calcium nitrate ($Ca(NO_3)_2 4H_2O$)	12.78	.....	12.78	21.14	.....	.....	.....	.....
Potassium sulphate (K_2SO_4)	.....	12.40	4.56	.....	16.96	.....	.....	.....
Sodium sulphate ($Na_2SO_4 10H_2O$)	3.16	19.86	2.44	.....	19.83	11.60	19.85	19.85
Calcium chlorid ($CaCl_2 6H_2O$)	.....	11.84	.....	.....	11.84	11.84	11.84	11.84
Magnesium chlorid ($MgCl_2 6H_2O$)	4.50	.....	4.50	4.50	.....	.....	.....	.....
Magnesium sulphate ($MgSO_4 7H_2O$)	.....	4.06	.....	2.66	4.06	4.06	4.06	4.06
Ferric tartrate ($Fe_2(C_4H_4O_6)_3 + H_2O$)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Calcium carbonate, precipitated, ($CaCO_3$)	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Water	100,000 cc.							

Precipitated calcium carbonate was used in all solutions, since preliminary tests showed that it measurably increased the growth of roots in certain incomplete solutions. Ferric tartrate was used as the source of iron, previous work having shown this to be an especially available form of iron in the presence of calcium carbonate. In all the nutrient solutions, except that lacking potassium, the bases were in the following proportions by weight: 1 Mg to 4 Ca to 5.3 Na to 14 K. In the solution lacking potassium the proportions were 1 Mg to 4 Ca to 6 Na.[1]

The nutrient solutions, with the exception of iron, were made up 18 hours before they were to be used. Ferric tartrate was added just before the solutions were used, since the availability of the iron in the solution decreases somewhat with time.

All the plants grown in the experiments were analyzed, by the usual analytical methods, for nitrogen, phosphoric acid, and potash.[2] The results reported are, in practically every case, the average of closely agreeing duplicate or triplicate determinations.

In each experiment one lot of plants was grown with all the roots in the complete nutrient solution, and another lot was grown with all the roots in the solution lacking nitrogen, phosphorus, and potassium. The normal, or maximum, assimilation of nitrogen, phosphorus, or potassium for any experiment was taken as the difference between the quantities present in these two lots of plants. The assimilations attained by plants having their roots divided between more or less complete solutions [3] were expressed relative to this maximum or normal assimilation.

In all the experiments 1 corn plant was grown in each double or triple flask, and 8 plants were taken as a unit, the units being duplicated for each separate treatment. There were, therefore, 16 plants which were treated alike in every case.

EXPERIMENTAL RESULTS

PLANTS GROWN WITH THEIR ROOTS EQUALLY DIVIDED BETWEEN TWO SOLUTIONS

EXPERIMENT 1.—The roots divided between a complete solution and a solution lacking two elements.

Since in this work only nitrogen, phosphorus, and potassium are varied, there are evidently only three possible pairs of solutions which

[1] The small amount of calcium resulting from the slight solubility of calcium carbonate was not considered in calculating these proportions.

[2] Nitrogen and potash were determined in the roots as well as in the stalks and leaves, since the roots could be washed free from nitrates and potash salts. Phosphoric acid, however, was determined only in the stalks and leaves, because it seemed probable that some precipitate of calcium or ferric phosphate might adhere to the roots, even after thorough washing.

[3] The amount of nitrogen, phosphoric acid, or potash assimilated by these plants was taken as the amount present in the plants minus the amount present in the plants grown in the solution lacking nitrogen, phosphorus, and potassium.

consist of a complete solution and one lacking in two elements. Data on the growth of plants in these three pairs of solutions are given in Table II, and data on the assimilation of nitrogen, phosphoric acid, and potash in Table III.

The plants having half their roots in incomplete solutions had slightly higher ratios of roots to tops than the control plants, which had all their roots in the complete solution.[1] It is also noticeable that plants 33 to 80 showed a greater growth of roots in the A flasks, which contained the complete solution, than in the B flasks containing the incomplete solutions.

The means of the amounts of nitrogen, phosphoric acid, and potash assimilated by plants 33 to 48, 49 to 64, and 65 to 80 were almost identical. The relative amounts of nitrogen, phosphoric acid, and potash assimilated by these three sets of plants varied, however, according to the character of the incomplete solution in flask B.

EXPERIMENT II.—The roots divided between a complete solution and a solution lacking three elements.

The data on growth and on the assimilation of nitrogen, phosphoric acid, and potash, along with the results of experiment VIII, are given in Tables XIV and XV.

While plants 49 to 64 had almost the same numbers of roots in the A and B flasks, nearly two-thirds of the total root growth was in the A flask containing the complete solution. The roots in the complete solution were much shorter and more bushy than those in the solution lacking nitrogen, phosphorus, and potassium.

The proportions in which nitrogen, phosphorus, and potassium were assimilated by plants 49 to 64 differed but little from the proportions assimilated by the control plants, 1 to 16, although the mean assimilation of these three elements by plants 49 to 64 was only about two-thirds that of the controls. It would be interesting to know whether assimilation would be cut to 50 per cent if one-half the roots were maintained in water containing no nutrients whatever—that is, no sodium, calcium, magnesium, iron, chlorids, and sulphates. Because of the difficulty of preparing sufficient quantities of suitable distilled water, this was not determined.

EXPERIMENT III.—The roots divided between two solutions, each of which lacked one element.

The three nutrient solutions lacking in nitrogen, phosphorus, and potassium, respectively, may be combined in three pairs. The growth made in these three pairs of solutions, and the analyses of the plants are shown in Tables IV and V. It will be noted that the growths made by the plants in each of the three pairs of incomplete solutions varied markedly. Roughly, the root to top ratios were inversely proportional to the growths of tops.

[1] The root to top ratio is taken as the weight of dry roots divided by the weight of dry leaves and stalks (tops).

TABLE II.—*Growth of plants in experiment I*

Plant No.	Nutrient solution.		Weight of stalks and leaves.			Number of roots.		Average oven-dry weight of roots.		Average oven-dry weight per root.		Ratio of roots to tops.	Percentage of total roots by weight.	
	A flasks.	B flasks.	Green.	Oven-dry.	Average oven-dry.	A flasks.	B flasks.	A flasks.	B flasks.	A flasks.	B flasks.		A flasks.	B flasks.
			Gm.	*Gm.*	*Gm.*			*Gm.*	*Gm.*	*Gm.*	*Gm.*			
1 to 8	Complete (NPK present)	Complete (NPK present)	241.78	19.01	19.31	88	98	2.39	2.40	0.0191	0.0248	0.252	50.5	49.5
9 to 16			247.8	19.6		97	98	2.38	2.28			.238		
17 to 24	NPK lacking	NPK lacking	15.85	2.26	2.18	58	63	.83	.79	.0126	.0133	.717	50.0	50.0
25 to 32			15.54	2.09		64	66	.83	.88			.818		
33 to 40	Complete (NPK present)	PK lacking (N present)	206.42	16.79	18.00	89	92	2.43	2.26	.0284	.0262	.279	52.0	48.0
41 to 48			229.43	19.21		90	87	2.69	2.45			.268		
49 to 56	Complete (NPK present)	NK lacking (P present)	218.56	18.11	17.85	96	100	2.77	1.88	.0289	.0193	.257	61.0	39.0
57 to 64			224.42	17.59		99	87	2.88	1.73			.262		
65 to 72	Complete (NPK present)	NP lacking (K present)	227.57	18.64	18.51	93	94	2.77	1.97	.0302	.0205	.254	58.3	41.7
73 to 80			225.8	18.38		93	102	2.84	2.05			.266		

TABLE III.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiment I*

Plant No.	Nutrient solution.		Nutrients present in oven-dry stalks and leaves.			Nutrients present in oven-dry roots.				Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to that of plants 1 to 16 taken as 100.
						Nitrogen (N).		Potash (K_2O).					
	A flasks.	B flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	A flasks.	B flasks.	A flasks.	B flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*				
1 to 16	Complete (NPK present)	Complete (NPK present)	3.81	1.53	8.18	2.47	2.47	5.37	5.37	100	100	100	100
17 to 32	NPK lacking	NPK lacking	1.21	.29	1.45	1.12	1.12	.98	.98	.....	.....	.....	.....
33 to 48	Complete (NPK present)	PK lacking (N present)	4.19	1.3	6.6	2.79	2.7	5.07	2.11	105	79	74	86
49 to 64	Complete (NPK present)	NK lacking (P present)	3.73	1.51	7.51	2.69	1.57	4.66	2.53	90	91	82	88
65 to 80	Complete (NPK present)	NP lacking (K present)	3.56	1.34	7.72	2.61	1.33	4.55	4.95	88	84	90	87

TABLE IV.—*Growth of plants in experiment III*

Plant No.	Nutrient solution.		Weight of stalks and leaves.			Number of roots.		Oven-dry weight of roots.		Average oven-dry weight per root.		Ratio of roots to tops.	Percentage of total roots by weight.	
	A flasks.	B flasks.	Green.	Oven-dry.	Average oven-dry.	A flasks.	B flasks.	A flasks.	B flasks.	A flasks.	B flasks.		A flasks.	B flasks.
			Gm.	*Gm.*	*Gm.*			*Gm.*	*Gm.*	*Gm.*	*Gm.*			
1 to 8	Complete (NPK present)..	Complete (NPK present)..	265. 99	21. 66	23. 56	100	100	2. 43	2. 50	0. 0256	0. 0255	0. 227	49. 2	50. 8
9 to 16			307. 7	25. 45		94	101	2. 52	2. 61			. 202		
17 to 24	NPK lacking.....	NPK lacking.....	11. 66	3. 36	3. 3	63	67	. 98	. 96	. 0153	. 0149	. 577	50. 2	49. 8
25 to 32			11. 35	3. 24		69	70	1. 04	1. 04			. 642		
33 to 40	N lacking (PK present)...	P lacking (NK present)...	125. 51	12. 21	12. 05	100	93	2. 22	2. 93	. 0220	. 0305	. 422	42. 5	57. 5
41 to 48			120. 17	11. 86		92	94	2. 00	2. 77			. 402		
49 to 56	N lacking (PK present)...	K lacking (NP present)...	166. 15	14. 87	16. 19	94	95	1. 85	2. 82	. 0196	. 0288	. 314	38. 9	61. 2
57 to 64			200. 22	17. 51		97	109	1. 88	3. 05			. 282		
65 to 72	K lacking (NP present)...	P lacking (NK present)...	220. 14	19. 89	20. 87	99	100	2. 62	2. 58	. 0278	. 0259	. 261	52. 6	47. 4
73 to 80			253. 13	21. 85		107	99	3. 09	2. 58			. 259		

TABLE V.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiment III*

Plant No.	Nutrient solution.		Nutrients present in oven-dry leaves and stalks.			Nutrients present in oven-dry roots.				Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to that of plants 1 to 16 taken as 100.
						Nitrogen (N).		Potash (K_2O).					
	A flasks.	B flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	A flasks.	B flasks.	A flasks.	B flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*				
1 to 16	Complete (NPK present)	Complete (NPK present)	3.73	1.62	7.69	2.58	2.58	5.66	5.66	100.0	100.0	100.0	100.0
17 to 32	NPK lacking	NPK lacking	1.17	.32	2.13	1.24	1.24	1.87	1.87	.....	.....	.....	.....
33 to 48	N lacking (PK present)	P lacking (NK present)	2.92	.62	6.44	1.43	2.61	4.58	5.41	42.6	17.3	46.3	35.4
49 to 64	N lacking (PK present)	K lacking (NP present)	3.56	1.63	5.08	1.86	2.97	4.84	1.89	67.2	68.3	43.3	59.6
65 to 80	K lacking (NP present)	P lacking (NK present)	3.91	1.4	5.78	3.01	2.8	1.88	5.26	96.4	75.9	64.8	79.0

In the two preceding experiments and in the work reported in the previous paper, where half the roots were in a complete solution, the lack of any one element did not significantly depress growth and assimilation more than the lack of another. In this experiment, however, where both the solutions were incomplete, growth and assimilation varied markedly according to which elements were supplied to only half the roots. A restriction of the supply of nitrogen and phosphorus to separate halves of the roots depressed growth and assimilation more than a similar restriction of the supply of nitrogen and potassium, or phosphorus and potassium.

The relative growths of roots in the A and B flasks, shown by the relative percentages of total roots by weight, in the two flasks is of interest. If the relative growths are indicative of the relative needs of the plant for the elements present in the solution, it would seem from the data on plants 33 to 48 that nitrogen is needed more than phosphorus; from plants 49 to 64, that nitrogen is needed much more than potassium; and from plants 65 to 80, that phosphorus is needed slightly more than potassium. The relative needs of the plants for these three elements may well hold for corn only and for this stage of growth of corn.

EXPERIMENT IV.—The roots divided between two solutions, one of which lacked one element and the other two elements.

The three nutrient solutions lacking nitrogen, phosphorus, and potassium, respectively, and the three solutions lacking nitrogen and phosphorus, nitrogen and potassium, and phosphorus and potassium, respectively, can be combined in nine pairs. Only three of the possible combinations, however, afford in the pair a complete nutrient solution. The effect of these three pairs of solutions on growth and the assimilation of nutrients are shown in Tables VI and VII.

The assumption made in the previous experiment, that corn needs nitrogen more than it needs phosphorus, and phosphorus slightly more than potassium during the period of growth covered by these tests, helps to explain the results obtained in this experiment as well as the results obtained in the succeeding ones. A second assumption, however, is needed—namely, the assimilation of nitrogen, phosphorus, or potassium is greater when these elements are absorbed from solutions containing two or all three of them than when they are absorbed from solutions containing only one of them.

This second assumption explains why plants 49 to 64 assimilated relatively more nitrogen than plants 33 to 48 and relatively more phosphorus than plants 65 to 80, also why plants 65 to 80 assimilated relatively more potassium than plants 49 to 64.

TABLE VI.—*Growth of plants in experiment IV*

Plant No.	Nutrient solution.		Weight of stalks and leaves.			Number of roots.		Oven-dry weight of roots.		Average oven-dry weight per root.		Ratio of roots to tops.	Percentage of total roots by weight.	
	A flasks.	B flasks.	Green.	Oven-dry.	Average oven-dry.	A flasks.	B flasks.	A flasks.	B flasks.	A flasks.	B flasks.		A flasks.	B flasks.
			Gm.	*Gm.*	*Gm.*			*Gm.*	*Gm.*	*Gm.*	*Gm.*			
1 to 8	Complete (NPK present)	Complete (NPK present)	227.22	18.21	19.65	91	80	2.46	2.13	0.0274	0.0249	0.251	53.4	46.6
9 to 16			242.37	21.09		89	94	2.47	2.21			.222		
17 to 24	NPK lacking	NPK lacking	18.57	2.44	2.54	55	50	.81	.79	.0148	.0158	.656	51.2	48.8
25 to 32			19.57	2.63		56	50	.85	.78			.62		
33 to 40	N lacking (PK present)	PK lacking (N present)	97.05	9.21	10.33	74	80	1.52	2.27	.0228	.0301	.412	42.5	57.5
41 to 48			133.32	11.45		82	80	2.04	2.55			.401		
49 to 56	K lacking (NP present)	NP lacking (K present)	174.36	14.75	14.01	90	89	3.00	1.85	.0303	.0199	.329	61.4	38.6
57 to 64			150.56	13.26		85	84	2.51	1.60			.31		
65 to 72	P lacking (NK present)	(NK lacking (P present)	116.65	10.9	10.92	81	85	2.70	1.94	.0305	.0223	.426	56.3	43.7
73 to 80			115.60	10.94		80	87	2.24	1.90			.379		

TABLE VII.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiment IV*

Plant No.	Nutrient solution.		Nutrients present in oven-dry stalks and leaves.			Nutrients present in oven-dry roots.				Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to that of plants 1 to 16 taken as 100.
						Nitrogen (N).		Potash (K_2O).					
	A flasks.	B flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	A flasks.	B flasks.	A flasks.	B flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*				
1 to 16	Complete (NPK present)	Complete (NPK present)	4.07	1.46	7.45	2.68	2.68	4.91	4.91	100.0	100.0	100.0	100.0
17 to 32	PK lacking	NPK lacking	1.13	.40	2.6	1.24	1.24	2.13	2.13	.....	.....	.....	.....
33 to 48	N lacking (PK present)	PK lacking (N present)	3.60	.83	5.03	1.72	2.92	4.77	1.78	48.5	28.0	35.8	37.4
49 to 64	K lacking (NP present)	NP lacking (K present)	3.64	1.31	4.44	3.00	1.64	1.78	4.24	65.4	62.7	41.8	56.6
65 to 80	P lacking (NK present)	NK lacking (P present)	3.00	.61	6.30	2.58	1.58	4.85	2.79	42.6	20.4	48.9	37.3

PLANTS GROWN WITH THEIR ROOTS EQUALLY DIVIDED AMONG THREE SOLUTIONS

It was of interest to see whether there would be a greater depression in growth and assimilation when the roots were divided among three solutions than when the roots were divided between two solutions.

EXPERIMENT V.—The roots divided among three solutions, each of which lacked one element.

In this experiment the roots were equally divided among three solutions which were lacking in nitrogen, phosphorus, and potassium, respectively. The data on growth and assimilation are given in Tables VIII and IX.

A division of the roots among three solutions, each of which lacked one element, depressed the growth by 13 per cent, and the mean assimilation of nitrogen, phosphorus, and potassium, by 32 per cent. In experiment III, where the roots were divided between two solutions, each also lacking in one element, the average depression in growth was 30 per cent and the average depression in the mean assimilation of nitrogen, phosphorus, and potassium was 42 per cent. The plants in experiment V may have had somewhat of an advantage over those in experiment III, since any one of the three elements was available to two-thirds of the roots in this experiment, while in experiment III two of the three elements were each available to only one-half of the roots.

The greatest growth of roots was made in the solution containing nitrogen and phosphorus (C flask), and the least growth took place in the solution containing phosphorus and potassium. These growths agree with the results shown in experiments III and IV.

It is interesting to note that the roots which were in the solution containing nitrogen and phosphorus were considerably richer in nitrogen than those which were in the solution containing nitrogen and potassium; also, the roots which were in the solution containing nitrogen and potassium were slightly richer in potassium than the roots which were in the solution containing phosphorus and potassium. These facts appear to confirm the results of the former experiments in showing that the need of the plants for nitrogen, phosphorus, and potassium is in the order given; and they also seem to indicate that the assimilation of one element from a solution facilitates the assimilation of another element from that solution. The roots in C flask, for example, were richer in nitrogen than those in B flask, because phosphorus is needed more than potassium, and on this account the assimilation of phosphorus aided the assimilation of nitrogen more than did the assimilation of potassium.[1]

EXPERIMENT VI.—The roots divided among three solutions, one of which lacked two elements and two of which each lacked one element.

[1] In this explanation it is assumed that a higher percentage of an element in the root at the time of analysis means a more active assimilation of this element by the root.

TABLE VIII.—*Growth of plants in experiment V*

Plant No.	Nutrient solution. A flasks.	B flasks.	C flasks.	Weight of stalks and leaves. Green.	Oven-dry.	Average oven-dry.	Number of roots. A flasks.	B flasks.	C flasks.	Oven-dry weight of roots. A flasks.	B flasks.	C flasks.	Average oven-dry weight per root. A flasks.	B flasks.	C flasks.	Percentage of total roots by weight. A flasks.	B flasks.	C flasks.	Ratio of roots to tops.
				Gm.	*Gm.*	*Gm.*				*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*				
1 to 8.	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	298.5	22.82	23.44	63	62	63	1.90	1.71	1.89	0.0318	0.0293	0.0297	35.6	31.1	33.3	0.241
9 to 16.				320.5	24.05		60	55	60	2.00	1.70	1.76							.227
17 to 24.	NPK lacking...	NPK lacking...	NPK lacking...	8.2	1.28	1.21	32	37	38	.44	.46	.46	.0147	.0129	.0127	33.8	33.8	32.4	1.063
25 to 32.				7.4	1.13		28	31	27	.44	.42	.38							1.097
33 to 40.	N lacking (PK present).	P lacking (NK present).	K lacking (NP present).	247.9	21.93	20.28	66	67	64	2.07	2.31	2.53	.0298	.0352	.0384	29.3	34.3	36.6	.315
41 to 48.				215.5	18.62		61	59	59	1.72	2.12	2.20							.324

TABLE IX.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiment V*

Plant No.	Nutrient solution. A flasks.	B flasks.	C flasks.	Nutrients present in oven-dry stalks and leaves. Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	Nutrients present in oven-dry roots. Nitrogen (N). A flasks.	B flasks.	C flasks.	Potash (K_2O). A flasks.	B flasks.	C flasks.	Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100. Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to that of plants 1 to 16 taken as 100.
				Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*				
1 to 16.	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	4.37	1.77	8.72	2.67	2.67	2.67	6.01	6.01	6.01	100	100	100	100
17 to 32.	NPK lacking.....	NPK lacking.....	NPK lacking.....	1.28	.77	.63	.....	.....	.....	.39	.39	.39	.....	.....	.....	.....
33 to 48.	N lacking (PK present).	P lacking (NK present).	K lacking (NP present).	3.89	1.21	6.18	1.65	2.45	3.16	5.21	5.61	2.43	81	58	64	68

When only nitrogen, phosphorus, and potassium are varied, there are nine possible combinations of two solutions lacking one element with one solution lacking two elements. The effects of two of these combinations on the growth of corn and the assimilation of nutrients are shown in Tables X and XI.

In the relative growths made by plants 33 to 48 and 49 to 64 there is further evidence that nitrogen is the element most needed by corn at this stage of growth. Plants 49 to 64 made the greater growth, doubtless because two-thirds of their roots were supplied with nitrogen, while only one-third of the roots of plants 33 to 48 were so supplied.

The relative growths of roots in the A, B, and C flasks present no unusual features; they are evidently dependent on the relative needs of the plant for nitrogen, phosphorus, and potassium, and on the proportions of the roots which were supplied with these three elements.

EXPERIMENT VII.—The roots divided among three solutions, one of which lacked one element and two of which lacked two elements.

Of the solutions named above, six combinations afford a complete nutrient solution; only two of the combinations, however, were tested. The results are shown in Tables XII and XIII.

Plants 49 to 64 contained more nitrogen and phosphorus than plants 33 to 48, since they had an advantage in assimilating these elements from the same solution.

EXPERIMENT VIII.—The roots divided among three solutions, each of which lacked two elements.

In this experiment the nitrogen was in one flask, the phosphorus in another, and the potassium in a third. The results are given in Tables XIV and XV.

The depressions in growth and assimilation and the increase in the root to top ratio were greater in this experiment than in any of the preceding ones.

The distribution of nitrogen and potassium between the roots and tops in this experiment may be of some significance; it seems to support the explanation offered later of the manner in which assimilation is depressed by a division of the roots between incomplete solutions. In the case of plants 33 to 48, the roots which were in the solution containing nitrogen have a higher percentage of nitrogen than the stalks and leaves; also, the roots from the potassium solution are richer in potassium than the tops. The control plants (1 to 16), however, have far higher percentages of nitrogen and potassium in the stalks and leaves than in the roots. In all the preceding experiments (except plants 49 to 64, experiment VII), where assimilation was depressed to a less extent than in this experiment, the stalks and leaves are richer in nitrogen and potassium than the roots.

TABLE X.—*Growth of plants in experiment VI*

Plant No.	Nutrient solution.			Weight of stalks and leaves.			Number of roots.			Oven-dry weight of roots.			Average oven-dry weight per root.			Percentage of total roots by weight.			Ratio of roots to tops.
	A flasks.	B flasks.	C flasks.	Green.	Oven-dry.	Average oven-dry.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	
				Gm.	*Gm.*	*Gm.*				*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*				
1 to 8	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	274.6	24.72	24.15	79	68	67	2.33	2.10	2.13	0.0316	0.0300	0.0301	36.3	32.1	32.7	0.265
9 to 16				262.7	23.57		71	75	79	2.40	2.21	2.26							.291
17 to 24	NPK lacking...	NPK lacking...	NPK lacking...	23.0	3.77	3.86	45	48	46	.69	.75	.75	.0136	.0144	.0147	31.2	34.4	34.4	.581
25 to 32				23.3	3.94		55	55	56	.67	.75	.74							.548
33 to 40	K lacking (NP present).	N lacking (PK present).	NP lacking (K present).	110.6	11.96	11.55	64	60	65	2.35	1.32	1.30	.035	.0218	.0212	44.2	27.9	27.9	.416
41 to 48				100.9	11.13		60	66	64	1.99	1.42	1.43							.435
49 to 56	K lacking (NP present).	P lacking (NK present).	NK lacking (P present).	175.5	18.39	18.36	72	71	68	2.35	2.64	1.57	.0362	.0368	.0232	36.9	39.0	24.2	.357
57 to 64				182.0	18.33		65	71	72	2.59	2.58	1.67							.373

TABLE XI.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiment VI*

Plant No.	Nutrient solution.			Nutrients present in the oven-dry stalks and leaves.			Nutrients present in oven-dry roots.						Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to that of plants 1 to 16 taken as 100.
							Nitrogen (N).			Potash (K_2O).						
	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
				Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*				
1 to 16	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	3.79	1.55	7.75	2.53	2.53	2.53	5.40	5.40	5.40	100	100	100	100
17 to 32	NPK lacking.....	NPK lacking.....	NPK lacking.....	1.07	.33	1.08	1.32	1.32	1.32	.82	.82	.82	.....	.....	.....	.....
33 to 48	K lacking (NP present).	N lacking (PK present).	NP lacking (K present).	2.85	1.06	4.41	2.68	1.48	1.45	1.85	2.69	4.33	35	30	27	31
49 to 64	K lacking (NP present).	P lacking (NK present).	NK lacking (P present).	3.31	.88	4.97	2.66	2.47	1.60	1.80	4.56	1.70	68	41	48	52

TABLE XII.—*Growth of plants in experiment VII*

Plant No.	Nutrient solution.			Weight of stalks and leaves.			Number of roots.			Oven-dry weight of roots.			Average oven-dry weight per root.			Percentage of total roots by weight.			Ratio of roots to tops.
	A flasks.	B flasks.	C flasks.	Green.	Oven-dry.	Average oven-dry.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	
				Gm.	*Gm.*	*Gm.*				*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*				
1 to 8	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	328.8	26.28	26.13	69	65	70	1.77	1.59	1.88	0.0257	0.0248	0.0257	33.0	32.8	34.2	0.199
9 to 16				315.4	25.98		70	71	68	1.62	1.78	1.66							.195
17 to 24	NPK lacking...	NPK lacking...	NPK lacking...	23.6	3.44	3.43	59	47	59	.78	.54	.84	.0136	.012	.0132	34.4	28.2	37.4	.628
25 to 32				25.3	3.41		47	51	61	.66	.63	.73							.592
33 to 40	N lacking (PK present).	PK lacking (N present).	NK lacking (P present).	152.8	14.29	13.74	70	66	73	1.64	2.16	1.62	.0238	.0311	.021	32.0	39.8	28.2	.379
41 to 48				137.8	13.19		66	64	62	1.60	1.88	1.23							.357
49 to 56	K lacking (NP present).	NP lacking (K present).	NK lacking (P present).	149.9	14.26	14.08	65	58	65	2.25	1.25	1.27	.0345	.0208	.0188	46.6	26.8	26.6	.334
57 to 64				147.7	13.90		64	66	70	2.22	1.33	1.29							.348

TABLE XIII.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiment VII*

Plant No.	Nutrient solution.			Nutrients present in the oven-dry stalks and leaves.			Nutrients present in the oven-dry roots.						Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to that of plants 1 to 16 taken as 100.
							Nitrogen (N).			Potash (K_2O).						
	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
				Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*				
1 to 16...	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	3.92	1.96	7.83	2.55	2.55	2.55	5.71	5.71	5.71	100	100	100	100
17 to 32..	NPK lacking.....	NPK lacking.....	NPK lacking.....	1.37	.29	1.24	1.37	1.37	1.37	.86	.86	.86	.....	.....	.....	.....
33 to 48..	N lacking (PK present).	PK lacking (N present).	NK lacking (P present).	3.25	.92	4.49	1.47	2.82	1.45	4.24	1.66	1.54	45	23	30	33
49 to 64..	K lacking (NP present).	NP lacking (K present).	NK lacking (P present).	3.45	1.43	3.75	3.04	1.62	1.62	1.49	4.44	1.39	49	38	26	38

TABLE XIV.—*Growth of plants in experiments II and VIII*

Plant No.	Nutrient solution.			Weight of stalks and leaves.			Number of roots.			Oven-dry weight of roots.			Average oven-dry weight per root.			Percentage of total roots by weight.			Ratio of roots to tops.
	A flasks.	B flasks.	C flasks.	Green.	Oven-dry.	Average oven-dry.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	
				Gm.	*Gm.*	*Gm.*				*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*				
1 to 8	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	437.4	35.85	33.22	75	70	74	3.01	2.65	2.88	0.0359	0.0354	0.0342	34.6	33.1	32.3	0.238
9 to 16				389.6	30.59		76	77	71	2.41	2.53	2.18							.233
17 to 24	NPK lacking	None	None	8.9	2.15	2.04	152	.....	.....	1.67	.....	.....	.0109	.....	.....	.....	.....	.....	.777
25 to 32				6.8	1.92		130	.....	.....	1.40	.....	.....							.729
33 to 40	PK lacking (N present).	NK lacking (P present).	NP lacking (K present).	98.1	11.60	11.38	73	65	64	2.40	1.82	1.95	.0373	.0258	.0272	42.3	27.7	30.1	.532
41 to 48				92.9	11.15		75	66	72	2.74	1.55	1.72							.539
49 to 56	Complete (NPK present).	NPK lacking	None	274.1	22.15	23.65	87	92	.....	4.28	2.49	.....	.0455	.0267	.....	62.7	37.4	.....	.296
57 to 64				287.2	24.44		106	103	.....	4.42	2.70	.....							.291

TABLE XV.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiments II and VIII*

Plant No.	Nutrient solution.			Nutrients present in the oven-dry stalks and leaves.			Nutrients present in the oven-dry roots.						Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to plants 1 to 16 taken as 100.
							Nitrogen (N).			Potash (K_2O).						
	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
				Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*				
1 to 16	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	3.98	1.59	8.08	2.46	2.46	2.46	5.53	5.53	5.53	100	100	100	100
17 to 34	NPK lacking.....	None.....	None.....	1.23	.52	.97	1.15	1.15	1.15	.66	.66	.66	.....	.....	.....	.....
35 to 48	PK lacking (N present).	NK lacking (P present).	NP lacking (K present).	2.42	.43	2.86	2.68	1.50	1.65	1.65	1.42	4.15	24	7	14	15
49 to 64	Complete (NPK present).	NPK lacking.....	None.....	3.81	1.41	7.46	2.61	1.59	.....	5.31	2.67	.....	69	62	66	66

TABLE XVI.—*Growth of plants in experiment IX*

Plant No.	Nutrient solution.			Weight of stalks and leaves.			Number of roots.			Oven-dry weight of roots.			Average oven-dry weight per root.			Percentage of total roots by weight.			Ratio of roots to tops.
	A flasks.	B flasks.	C flasks.	Green.	Oven-dry.	Average oven-dry.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	
				Gm.	*Gm.*	*Gm.*				*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*				
1 to 8	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	318.7	26.51	26.72	71	75	63	1.87	2.15	2.09	0.0267	0.0279	0.0298	31.5	35.3	33.3	.023
9 to 16				320.9	26.92		71	78	73	1.92	2.10	1.92							.221
17 to 24	NPK lacking...	NPK lacking..	NPK lacking..	14.5	2.41	2.49	50	45	54	.60	.50	.65	.0122	.013	.0128	32.4	33.5	34.1	.726
25 to 32				15.7	2.57		43	45	40	.53	.67	.54							.677
33 to 40	Complete (NPK present).	N lacking (PK present).	K lacking (NP present).	250.6	22.42	22.91	70	62	66	2.29	1.43	2.05	.0293	.0215	.029	38.3	25.5	36.1	.257
41 to 48				270.0	23.40		71	66	69	1.83	1.31	1.85							.209
49 to 56	P lacking (NK present).	N lacking (PK present).	PK lacking (N present).	168.8	17.43	17.28	74	70	72	2.42	1.88	2.37	.0333	.0276	.0309	36.6	29.3	34.1	.382
57 to 64				167.9	17.13		71	70	74	2.4	1.98	2.13							.38
65 to 72	P lacking (NK present).	NK lacking (P present).	PK lacking (N present).	107.7	12.48	12.61	70	62	64	1.78	1.43	1.59	.0286	.0231	.0248	37.9	28.4	33.7	.385
73 to 80				107.8	12.73		65	59	70	1.96	1.36	1.73							.397

TABLE XVII.—*Nitrogen, phosphoric acid, and potash absorbed by plants in experiment IX*

Plant No.	Nutrient solution.			Nutrients present in the oven-dry stalks and leaves.			Nutrients present in the oven-dry roots.						Absorption of nutrients relative to amounts assimilated by plants 1 to 16 taken as 100.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to that of plants 1 to 16 taken as 100.
							Nitrogen (N).			Potash (K_2O).						
	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
				Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*				
1 to 16	Complete (NPK present).	Complete (NPK present).	Complete (NPK present).	3.41	1.54	7.23	2.36	2.36	2.36	4.51	4.51	4.51	100	100	100	100
17 to 32	NPK lacking....	NPK lacking.....	NPK lacking.....	1.02	.55	1.09	1.07	1.07	1.07	.70	.70	.70	.....	.....	.....	.....
33 to 48	Complete (NPK present).	N lacking (PK present).	K lacking (NP present).	3.33	1.38	5.83	2.37	1.55	2.32	3.99	4.43	1.77	83	76	68	76
49 to 64	P lacking (PK present).	N lacking (PK present).	PK lacking (N present),	2.58	.47	4.86	2.05	1.36	2.10	4.28	3.82	1.85	52	17	46	38
65 to 80	P lacking (NK present).	NK lacking, P present).	PK lacking (N present).	2.21	.39	3.87	2.08	1.36	2.01	3.89	1.76	1.86	32	9	27	23

EXPERIMENT IX.—The roots divided among three solutions. Various combinations of solutions compared.

It is quite evident from a comparison of the results of the preceding experiments that the depressions in growth and absorption were roughly proportional to the incompleteness of the solutions afforded the roots. This is further demonstrated in the present experiment where solutions lacking in different numbers of nutrients are directly compared. The results are given in Tables XVI and XVII.

SUMMARY OF EXPERIMENTAL RESULTS

The important experimental data of all the tests are gathered together in Table XVIII. Since only nitrogen, phosphorus, and potassium were varied in these tests (calcium, magnesium, sodium, iron, sulphate, and chlorid being present in all the solutions), the different nutrient solutions are designated in Table XVIII by their content of nitrogen, phosphorus, and potassium. NP, for example, refers to the solution lacking potassium, and NPK stands for the complete solution, while O represents the solution lacking the three elements. The different combinations of solutions tested are arranged in Table XVIII with the idea of facilitating comparisons.

TABLE XVIII.—*Summary of results of experiments I to IX*

Experiment No.	Solution.			Weight per root.			Percentage of total roots by weight.		
	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.	A flasks.	B flasks.	C flasks.
				Gm.	*Gm.*	*Gm.*			
I	NPK	N		0.0284	0.0262		52.0	48.0	
I	NPK	P		.0289	.0193		61.0	39.0	
I	NPK	K		.0302	.0205		58.3	41.7	
II	NPK	O		.0455	.0267		62.7	37.4	
III	PK	NK		.022	.0305		42.5	57.5	
III	PK	NP		.0196	.0288		38.9	61.2	
III	NP	NK		.0278	.0259		52.6	47.4	
IV	PK	N		.0228	.0301		42.5	57.5	
IV	NP	K		.0303	.0199		61.4	38.6	
IV	NK	P		.0305	.0223		56.3	43.7	
IX	NPK	PK	NP	.0293	.0215	0.029	38.3	25.5	36.1
V	PK	NK	NP	.0298	.0352	.0384	29.3	34.3	36.6
IX	NK	PK	N	.0333	.0376	.0309	36.6	29.3	34.1
VI	NP	PK	K	.035	.0218	.0212	44.2	27.9	27.9
VI	NP	NK	P	.0362	.0368	.0232	36.9	39.0	24.2
IX	NK	P	N	.0286	.0231	.0248	37.9	28.4	33.7
VII	PK	N	P	.0238	.0311	.021	32.0	39.8	28.2
VII	NP	K	P	.0234	.0308	.0188	46.6	26.8	26.6
VIII	N	P	K	.0373	.0258	.0272	42.3	27.7	30.1

TABLE XVIII.—*Summary of results of experiments I to IX*—Continued

Experiment No.	Ratio of roots to tops relative to that of controls taken as 100.[a]	Assimilation relative to that of controls taken as 100.[a]			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O), relative to that of controls taken as 100.[a]
		Nitrogen (N).	Phosphoric acid (P_2O_5).	Potash (K_2O).	
I	112	105	79	74	86
I	106	90	91	82	88
I	106	88	84	90	87
II	124	69	62	66	66
III	192	43	17	46	35
III	139	67	68	43	59
III	121	96	76	65	79
IV	180	49	28	36	37
IV	144	65	63	42	57
IV	170	43	20	49	37
IX	103	83	76	68	76
V	137	81	58	64	68
IX	169	52	17	46	38
VI	153	35	30	27	31
VI	131	68	41	48	52
IX	173	32	9	27	23
VII	187	45	23	30	33
VII	173	49	38	26	38
VIII	227	24	7	14	15

[a] The control plants were those grown with all their roots in the complete solution.

The more important facts established in the preceding experiments are as follows:

1. Depressions in growth and in assimilation of nutrients were roughly proportional to the incompleteness of the solutions afforded the roots, or, in other words, proportional to the extent the nutrients were restricted to separate portions of the roots.

2. Assimilation did not diminish with increasing subdivision of the roots among different solutions, unless the division entailed increased localization of the supply of the various nutrients.

3. The more growth and assimilation were depressed by division of the roots among incomplete solutions the higher was the ratio of root growth to top growth. However, the relation between diminution in assimilation and increase in the root to top ratio was not quantitatively proportional.

4. When different portions of the roots were supplied with different nutrient solutions, the roots in the more complete solutions generally made the greater growth and had a more bushy habit of growth. In the solutions lacking two elements the main roots were longer than in the more complete solutions, the lateral roots were fewer, and the laterals were farther apart on the main root.

5. When the roots were divided between solutions that were lacking in the same number of nutrients, root growth was greatest in the solution containing nitrogen.

6. The relative, as well as the absolute, root growth made in any solution, however, depended on the character of the solution in which the remainder of the roots were growing.

7. When the roots were divided among three incomplete solutions, each of which lacked either one or two of the elements, nitrogen, phosphorus, and potassium, the amount of nitrogen assimilated approached the normal assimilation of nitrogen—that is, the assimilation of plants with all their roots in a complete solution—considerably nearer than the amount of potassium assimilated approached the normal assimilation of potassium; also, potassium was assimilated to a very slightly nearer normal extent than phosphorus. This fact doubtless would not hold for all plants or for all stages of growth.

DISCUSSION OF RESULTS

The rate at which nutrient ions are assimilated by the plant is doubtless dependent upon the rates of absorption, translocation within the plant, and utilization, or the rate at which the ions are built up into complex compounds. These three rates of absorption, translocation, and utilization are, of course, mutually dependent, a reduction in any one reducing the other two.

The inability of a plant to effect a maximum assimilation of an ion which is supplied to only a portion of the roots evidently is not due to the root cells being unable to absorb this ion with sufficient rapidity. Data presented in the previous paper showed that roots could increase their rate of absorption very markedly. When only one-fourth of a plant's roots were supplied with nitrates, these roots absorbed nitrogen 2.26 times as rapidly as the roots of plants which were completely supplied with nitrates.

The diminished assimilation of nutrients when the roots are divided between incomplete solutions is more probably due to a diminution in the rate at which the nutrients are translocated to the cells where they are utilized. Although it is not known exactly how the ions are translocated, a rough explanation can be given of how the transference of ions in the vegetative part of the plant would be slowed down by absorption of the different nutrients by separate roots.

When, for instance, the nitrogen, phosphorus, and potassium are confined to separate roots (as in experiment VIII), there is an unusual transference of nitrogen to the roots in the phosphorus and potassium solutions, and an extraordinary transference of phosphorus to the roots in the nitrogen and potassium solutions, etc. The extra work of this unusual transference of nutrients, however, is hardly sufficient to account for the diminished assimilation.

Probably the chief inhibition to translocation arises from the fact that nitrogen, phosphorus, and potassium are more or less scattered, as it were, in different parts of the plant, as a result of having been absorbed by different roots. Doubtless they are, for the most part, in different fibrovascular bundles and must be translocated by separate paths, instead of all together, to the cells where they are to be utilized. A cell, for example, which is adjacent to a fibrovascular bundle that emanates from a root in the phosphorus solution can secure phosphorus at once, but the nitrogen and potassium have to be transported from other centers in the plant. No more phosphorus can be assimilated by this cell until the nitrogen and potassium are secured. Under normal conditions the three elements would be obtained from the same fibrovascular bundle and they would be assimilated more quickly.

The foregoing suggestion concerning the manner in which translocation and assimilation may be depressed by a division of the roots between incomplete solutions seems to explain facts 1 and 2 of the summary given on page 568. The view that diminished assimilation is due to slow translocation rather than to a reduced power of absorption is also supported by the results of experiment VIII. In this experiment, where the assimilation was unusually low, the amounts of nitrogen and potassium in the roots were unusually large in proportion to the percentages in the tops.

It follows from the explanation of the way assimilation is depressed that when roots are in different incomplete nutrient solutions, slowness in the translocation or assimilation of one element reduces the rate of assimilation of other elements which are confined to other roots. This suggests a method, described below, for calculating the mean assimilation of those elements which are confined to certain roots, by making use of data presented by the authors in the previous paper.

This earlier paper contains a graph giving a curve which shows the assimilation of an element (relative to the normal) that would be attained when any fraction of the roots were deprived of one element, the remainder of the roots being in a complete solution. This curve, experimentally determined (reproduced in the present paper in fig. 1), agreed very closely with Mitscherlich's law of minimum.

While this curve is directly valid only for the condition where part of the roots are in a complete solution and part in a solution lacking one element, nevertheless, by utilizing values obtained from it, one can calculate fairly closely the mean of the amounts of nitrogen, phosphorus, and potassium that will be assimilated when the roots are in two or three different solutions each of which is lacking in one or more of these elements. It is only necessary to take from the curve the assimilations of nitrogen, phosphorous, and potassium (expressed as percentages of the normal assimilations) which correspond to the respective fractions of the roots supplied with these elements and then multiply the percentages

of assimilation together. The result will agree fairly well with the mean of the amounts of nitrogen, phosphorus, and potassium actually assimilated. In experiment VIII, for instance, 42.3 per cent of the roots were supplied with nitrogen, 27.7 per cent were supplied with phosphorus, and 30.1 per cent were supplied with potassium. Under the conditions for which the curve is valid, the assimilations corresponding to these fractions of the roots would be 67, 56, and 57 per cent, respectively. These percentages multiplied together give 21 per cent, and the actual

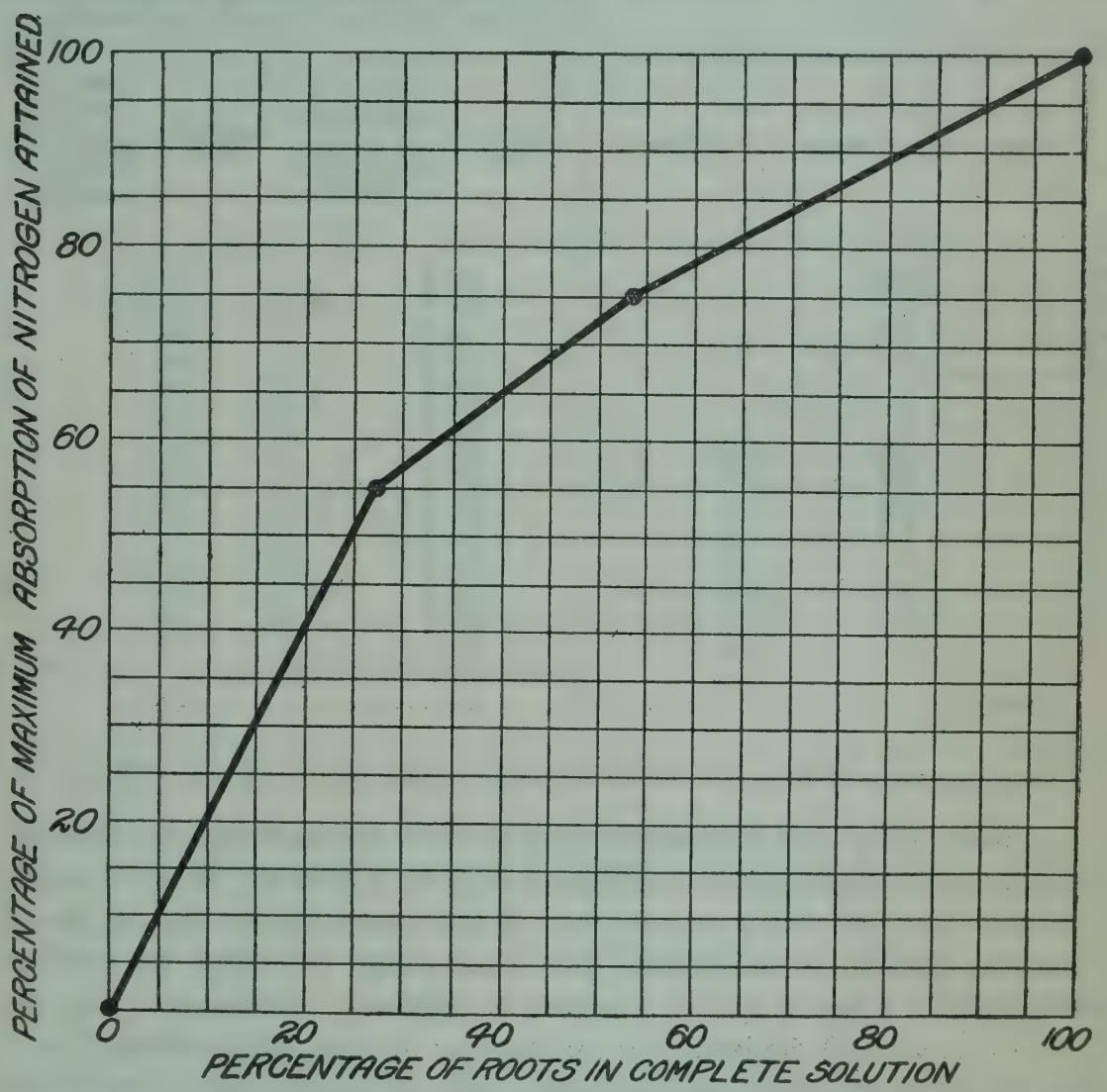

FIG. 1.—Relation between percentage of roots supplied with nitrogen and percentage of maximum absorption.

mean assimilation in this experiment was 15 per cent of that of the normal or control plants.

In Table XIX are given the actual and calculated values for the mean assimilations of nitrogen, phosphorous, and potassium in experiments where the roots were in incomplete solutions.

The agreement between the calculated and actual values is, for the most part, fairly close for work of this nature. The average values for a general type of combination of solutions, such as two solutions lacking

one element combined with one solution lacking one element, agree more closely than the values for any one combination of specific solutions. This is because the method of calculation does not take into account certain less important factors, such as the apparent greater need of the plant for nitrogen than for phosphorus or potassium.

TABLE XIX.—*Actual and calculated values of mean assimilations of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O)*

Solution.			Mean assimilation of nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) relative to the normal taken as 100.[a]			
A flasks.	B flasks.	C flasks.	Actual value.	Average of actual values for similar combinations of solutions.	Calculated values.	Average of calculated values for similar combinations of solutions.
PK	NK		35	58	52	53
PK	NP		59		52	
NP	NK		79		54	
PK	N		37	44	36	40
NP	K		57		42	
NK	P		37		42	
PK	NK	NP	68		57	
NK	PK	N	38	40	40	44
NP	PK	K	31		46	
NP	NK	P	52		46	
NK	P	N	23	31	30	32
PK	N	P	33		31	
NP	K	P	38		34	
N	P	K	15		21	
Average			43		42	

[a] The normal assimilation is that of the control plants grown with all their roots in the complete solution.

It is interesting to note that the way growth and assimilation diminish with increasing localization of different nutrients does not follow the law of minimum. According to any one of the formulations of the law of minimum, growth is not much less when three elements are equally deficient than when only one element is deficient. However, there are many experimental deviations from the law as usually formulated, and these results suggest an explanation for some of the apparent exceptions.

The fact that in this work the ratio of roots to tops increased as assimilation diminished may mean simply that a reduced assimilation of nutrients depresses the growth of roots less than it does the growth of tops. It may be, however, that a diminished assimilation of nutrients is directly stimulating to root growth. The growth of roots depends, of course, among other things, on the amount of organic material transported from the tops. Whether organic compounds are transferred to different parts of the leaves and stalks or to the roots may well be governed in part by the rate nutrient ions are utilized in the tops. When utilization is slow,

organic compounds may be transported to the roots; and when utilization is rapid, movement to the roots may be retarded. On such a basis, it might be said that slow utilization of nutrient ions, due either to a deficiency in the supply or to a reduced rate of translocation in the plant, would be stimulating to root growth. This, of course, applies to total root growth.

The relative amounts of root growth made in the different solutions (when the roots were divided) would depend on the relative needs of the plant for the nutrients present in the solutions, since the organic compounds would evidently be most quickly utilized in those roots which contained the greatest number of the essential ions.

REDUCTION IN THE STRENGTH OF THE MERCURIC-CHLORID SOLUTION USED FOR DISINFECTING SWEET POTATOES

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INTRODUCTION

The disinfection of seed sweet potatoes with mercuric-chlorid solution ($HgCl_2$) before bedding for the prevention of certain diseases has become a common practice since it was first recommended by Harter [2] in 1913. All investigators working on sweet potatoes recommend in general the same method of treatment, although they differ slightly on certain details of procedure. In fact it has never been satisfactorily determined how many bushels can be treated in a solution of 1 to 1,000 mercuric chlorid before it becomes so reduced in strength as to be no longer effective.

Some determinations have been made of the amount of mercuric chlorid removed from the solutions used for treating Irish potatoes, the results of which, so far as the writer is aware, have never been published.[3] However, the data thus obtained have influenced some investigators studying Irish potato diseases to recommend either that the solution be discarded after having been used three or four times or that a definite quantity of mercuric chlorid be added from time to time to bring the solution back to approximately its original strength.

Orton [4] states that the solution used for disinfecting Irish potatoes grows rapidly weaker, even losing as much as one-fourth of its strength during a single treatment. He recommends that this loss be offset by the addition of 1 ounce of mercuric chlorid to each barrel of solution after one batch of potatoes has been treated. It is suggested that after repeating this process three or four times the solution be discarded. This method of procedure for the treatment of Irish potatoes seems to be pretty generally agreed upon, since it is sanctioned by pathologists from several of the leading Irish potato growing States.

It has been assumed that Irish and sweet potatoes probably produce a similar change in the solution in which they are treated. Hence,

[1] The writer is indebted to Dr. L. L. Harter for valuable suggestions and criticisms during the progress of the work reported in this paper.

[2] HARTER, L. L. CONTROL OF THE BLACK-ROT AND STEM-ROT OF THE SWEET POTATO. *In* U. S. Dept. Agr. Bur. Plant Indus. Circ. 114, p. 15–18. 1912.

[3] Since this article was submitted for publication results of a similar nature have been published. (BRANN, J. W., and VAUGHAN, R. E. POTATO SCAB. Wis. Agr Exp. Sta. Bul. 331, 27, p., 11 fig. 1921.)

[4] ORTON, W. A. SELECTION AND TREATMENT OF SEED POTATOES TO AVOID DISEASES. U. S. Dept. Agr. Bur. Plant Indus. C. T., and F. C. D. Circ. 3, 8 p., 2 fig. 1919.

recommendations have been made that the solution be discarded after 3 or 4 bushels of sweet potatoes were treated. Harter [1] states that the solution should not be used more than two or three times, since it loses its efficiency by repeated use. On the other hand, Taubenhaus [2] advises that the solution be used until exhausted.

The experiments reported in this paper were designed (1) to determine the rate of reduction in the strength of the mercuric-chlorid solution used for treating sweet potatoes and (2) to work out a method of procedure for growers whereby they might be reasonably sure of maintaining the solution at approximately its original strength with the least expenditure of time and money.

METHODS

The sweet potatoes used in these experiments were of the Yellow Jersey variety, grown on a sandy soil and stored in crates. The potatoes were sorted and placed in bushel hampers in which they were treated. In this manner the amount of dirt added to the solution was reduced to a minimum—that actually clinging to the potatoes. The solution was made up in a clean 50-gallon oak barrel by adding 4 ounces of mercuric chlorid previously dissolved in warm water to 32 gallons of water. Samples of the solution were taken for analysis both before being used and from time to time as indicated in the tables. In order to keep the solution as nearly as possible at its original strength, ¾ ounce of mercuric chlorid was added after each 10 bushels of potatoes were treated. In the experiments where the amount of mercuric chlorid removed by a specific substance, such as dirt, bags, hamper, etc., was being tested, the receptacle used for the solution was of such a nature that no reaction would take place between it and the chemical. In all such experiments, except where the hamper and the 30 pounds of potatoes were treated, a 2½-gallon porcelain bucket containing 2 gallons of solution was used, and in those two cases a 10-gallon glazed earthen jar with 7 gallons of solution was used.

The samples were analyzed the same day the treatments were made, with the exception of those in the experiments, the results of which are recorded in Table I. These were made the following day. All analyses were made by the volumetric method described by Jamieson.[3] In brief, the method consists of precipitating the mercury as mercury zinc thiocyanate, collecting the precipitate on a filter, and titrating it with a standard solution of potassium iodate (KIO_3) in the presence of strong hydrochloric acid, using chloroform as an indicator.

[1] HARTER, L. L. SWEET POTATO DISEASES. U. S. Dept. Agr. Farmers' Bul. 714, 26 p., 21 fig. 1916.

[2] TAUBENHAUS, J. J. FIELD DISEASES OF THE SWEET POTATO IN TEXAS. Tex. Agr. Exp. Sta. Bul. 249, 22 p., 34 fig. 1919.

[3] JAMIESON, George S. THE GRAVIMETRIC AND VOLUMETRIC DETERMINATION OF MERCURY PRECIPITATED AS MERCURY ZINC THIOCYANATE. *In* Jour. Indus. Engin. Chem., v. 11, no. 4, p. 296–297. 1919

Duplicate samples of 100 cc. each were placed in clean 250-cc. beakers, to which were added 25 cc. of the precipitating reagent, which consists of 39 gm. of ammonium thiocyanate and 29 gm. of zinc sulphate per liter. The solutions were vibrated by striking the sides of the beakers with a stirring rod to facilitate the separation of the crystals. After five minutes they were stirred rapidly for one minute and then allowed to stand for one hour or longer. Each solution was then filtered through a No. 42, 9-cm., chemically prepared Whatman's filter paper by the aid of gentle suction. The filter paper and precipitate were washed thoroughly with a solution made by adding 10 cc. of the thiocyanate reagent to 450 cc. of water. When the filter paper was thoroughly drained it was removed to a glass-stoppered titration bottle. To each bottle was then added a mixture consisting of 35 cc. of concentrated hydrochloric acid, 10 cc. of water, and 7 cc. of chloroform. This solution was then immediately titrated against a standard potassium-iodate solution containing 19.2191 gm. of potassium iodate per liter. The iodin liberated gave a brilliant red color to the chloroform, which changed to a pink and disappeared altogether when the end point was reached. The chloroform settled to the bottom of the bottle, and even in the presence of a considerable amount of dirt the end point was not obscured. Hence it was never necessary to filter the solutions to be tested.

In previous years attempts were made to carry out experiments of a similar nature, using the potassium-cyanid method for the determination of mercury. This method consists in titrating the mercuric-chlorid solution against a standard potassium-cyanid solution, using phenolphthalein as an indicator. In order to detect the end point, it was necessary to have a clear, colorless solution, which was often impracticable, especially since every attempt made to clear the solutions resulted in a decrease in the amount of mercury. The strength of a clean, standard solution of mercuric chlorid filtered through a single filter paper of various grades was reduced 1 to 3 per cent, and when two filter papers were used the concentration was lessened by as much as 6 per cent. In view of these facts a method had to be found in which filtering was not necessary. In one instance an attempt was made to determine the amount of mercury removed by a gunny sack by the use of potassium cyanid, but the analysis showed a larger amount of mercuric chlorid present in the solution after soaking the sack for 10 minutes than at the start. These results indicate that some substance present in the sack reacted with the potassium cyanid. No such inconsistencies were found in any of the analyses made by the Jamieson method. Some variation in the duplicate titrations occurred, but this was never more than one-half of 1 per cent and was usually less. The figures given in the tables are calculated from the averages of two or more titrations.

EXPERIMENTAL DATA

AMOUNT OF MERCURIC CHLORID REMOVED FROM THE SOLUTION DURING THE ORDINARY COMMERCIAL TREATMENT OF SEED SWEET POTATOES

In order to determine the amount of mercuric chlorid removed from the solution in which seed sweet potatoes were treated, two duplicate experiments were conducted, the results of which are recorded in Table I. A sample of the solution was taken in each case before any potatoes were added, and others were taken after each 5 bushels treated. After two 5-bushel lots were treated ¾ ounce of mercuric chlorid dissolved in water was added, the solution was stirred thoroughly, and another sample was taken. After four 5-bushel lots of potatoes were treated the solutions were made up to their original volume.

For the sake of convenience in presenting the data in the tables the 1-to-1,000 mercuric-chlorid solution is considered equal to 100 per cent, and all of the other concentrations are calculated on the same basis.

TABLE I.—*Percentage of mercuric chlorid removed from solution by treating 5 bushels of sweet potatoes in 32 gallons of solution for five minutes*

Number of bushels treated.	Amount of $HgCl_2$ added.	Percentage of $HgCl_2$ present in solution.		Percentage of $HgCl_2$ removed by each 5 bushels of potatoes treated.	
		Experiment 1.	Experiment 2.	Experiment 1.	Experiment 2.
	Ounces.				
0	.....	96.6	95.6	.....	.....
5	.....	90.3	89.9	6.3	5.7
5	.....	84.2	86.5	6.1	3.4
0	¾	103.1	102.3	.....	.....
5	.....	97.0	100.3	6.1	2.0
5	.....	89.1	95.5	7.9	4.8
0	¾[a]	90.9	105.2	.....	.....
5	.....	87.1	103.4	3.8	1.8
Averages[b]	.....	.....	.....	6.04	3.54

[a] Water was added to restore the solution to its original volume. This required 5 gallons in experiment 1 and 2½ gallons in experiment 2.

[b] Average for the two experiments=4.79 per cent.

An examination of Table I shows that in neither experiment was the original solution of a 1 to 1,000 strength. However, no attempt was made to measure the water accurately, but an effort was made to duplicate the method which a sweet-potato grower would probably use. The results of the analyses in Table I, experiment 1 (treatment 5 minutes), show that the first 5 bushels of potatoes treated reduced the strength of the solution 6.3 per cent, the second 5 bushels 6.1 per cent, the third 6.1 per cent, the fourth 7.9 per cent, and the fifth 3.8 per cent, making an average reduction of 6.04 per cent for each 5 bushels of potatoes treated. In experiment 2 (treatment 10 minutes) the first 5 bushels reduced the

strength of mercuric chlorid 5.7 per cent, the second 3.4 per cent, the third 2 per cent, the fourth 4.8 per cent, and the fifth 1.8 per cent, or an average reduction of 3.54 per cent. The potatoes used in the latter experiment had been employed in previous experiments of another nature, and some of them had had a considerable part of the dirt removed by handling or by being dipped into water. The potatoes used in the first experiment, however, had been selected for seed in the autumn, were stored in crates, and had not been handled until they were placed in the baskets in which they were treated. Nevertheless, data to be presented later will make it clear that a considerable amount of variation is to be expected.

In both experiments ¾ ounce of mercuric chlorid was added after each 10 bushels treated, in an endeavor to bring the solution up to its original strength. No data were at hand from which the correct amount of mercuric chlorid to be added could be calculated. It will be noted that in all but one case—namely, in the first experiment—after 20 bushels were treated, the amount of mercuric chlorid added was more than enough to return the solution to its original concentration.

Table I shows that within the limits of these experiments the addition of ¾ ounce of mercuric chlorid after 10 bushels of potatoes were treated sufficed to keep the disinfectant up to a strength where it was probably effective for the treatment of a total of 20 bushels without the addition of water. However, the thoroughness with which the potatoes are drained on removal from the barrel determines to a considerable extent the amount of water it is necessary to add. If reasonable care is exercised 20 bushels may be treated without the addition of water.

MERCURIC CHLORID REMOVED BY CLEAN SWEET POTATOES

The results obtained by the analyses of the solutions used for disinfecting sweet potatoes according to the method already described show that a considerable amount of the mercuric chlorid was removed. However, the results give no clue as to what was responsible for the change produced in the concentration of the solution. An attempt was next made to determine to what extent the potatoes, the dirt, and the container were responsible for the reduction in the strength of the mercuric chlorid.

The ability of potatoes, which were without doubt the most bulky substance added to the solution, to take up mercury was tested first. Six pounds of sweet potatoes from the storage house were washed free from dirt in running water, dried, and then immersed in 2 gallons of a solution of mercuric chlorid for 1½ hours, a sample being taken before the potatoes were added and again at various intervals, as indicated in Table II. The solutions of mercuric chlorid used in all of the following experiments were prepared separately.

TABLE II.—*Percentage of mercuric chlorid removed from solution by treating 6 pounds of washed sweet potatoes in 2 gallons of solution*

Length of treatment.	Percentage of $HgCl_2$ present in solution.		Percentage of $HgCl_2$ removed from solution.	
	Experiment 1.	Experiment 2.	Experiment 1.	Experiment 2.
Control [a]	98.2	100.0	.....	.....
5 minutes	97.2	98.8	1.0	1.2
10 minutes	96.9	98.3	1.3	1.7
30 minutes	96.8	98.0	1.4	2.0
60 minutes	97.1	.....	1.1	.....
90 minutes	97.2	98.0	1.0	2.0

[a] Solution unused.

The control in Table II and in other tables to follow shows the concentration of the solution before the potatoes were added. In experiment 1 there was a decrease of 1 per cent in the amount of mercuric chlorid present after 5 minutes and a decrease of 1.3 per cent, 1.4 per cent, 1.1 per cent, and 1 per cent after 10, 30, 60, and 90 minutes, respectively. These results may seem inconsistent, but as a matter of fact the variations are well within the limits of experimental error, since a difference of 0.3 per cent is equivalent to only 0.05 cc. of potassium iodate. It is evident, then, that the potatoes removed approximately 1 per cent of the mercuric chlorid during the first 5 minutes and no appreciable amount thereafter, at least up to 1½ hours.

Experiment 2 was an exact duplicate of experiment 1, except that no sample was taken at the end of 60 minutes. Here there was a reduction in mercuric chlorid of 1.2 per cent after 5 minutes, 1.7 per cent after 10 minutes, and 2 per cent thereafter up to the end of the experiment. Again the greatest decrease occurred during the first 5 minutes, followed, however, by a further slight reduction during the next 5 minutes. The reduction of 0.3 per cent after 30 minutes is well within the limits of experimental error.

These two experiments show that the greatest reduction in the concentration of the mercuric chlorid took place during the first 5 minutes of treatment. However, it was thought desirable to conduct a similar experiment on a larger scale. Accordingly 30 pounds of washed potatoes were treated in 7 gallons of mercuric-chlorid solution, and samples were taken for analysis, the results of which are given in Table III.

The results show a reduction of 2.4 per cent in the amount of mercuric chlorid at the end of the first five minutes, the concentration remaining practically constant thereafter. These experiments show that the sweet potatoes removed some of the mercuric chlorid from the solution and that by far the largest part was taken out during the first five minutes.

TABLE III.—*Percentage of mercuric chlorid removed from solution by treating 30 pounds of washed sweet potatoes in 7 gallons of solution*

Length of treatment.	Percentage of $HgCl_2$ present in solution.	Percentage of $HgCl_2$ removed from solution.
Control [a]	99.6	.....
5 minutes	97.2	2.4
10 minutes	97.4	2.2
30 minutes	97.2	2.4
60 minutes	97.2	2.4
90 minutes	97.0	2.6

[a] Solution unused.

A COMPARISON OF THE AMOUNT OF MERCURIC CHLORID REMOVED BY IRISH AND SWEET POTATOES

Since it was customary to recommend that the solution used for treating sweet potatoes be discarded after 3 or 4 bushels had been treated on the assumption that sweet potatoes would remove from the solution about the same amount of mercuric chlorid as Irish potatoes, a comparative test with Irish potatoes was made. Six pounds of Irish potatoes of the Burbank variety were washed and treated, a sample being taken before disinfecting the potatoes and at stated intervals thereafter, as shown in Table IV.

TABLE IV.—*Percentage of mercuric chlorid removed from solution by treating 6 pounds of washed Irish potatoes in 2 gallons of solution*

Length of treatment.	Percentage of $HgCl_2$ present in solution.	Percentage of $HgCl_2$ removed from solution.
Control [a]	97.2	.....
5 minutes	96.4	0.8
10 minutes	96.4	.8
30 minutes	96.4	.8
60 minutes	96.4	.8
90 minutes	96.0	1.2

[a] Solution unused.

The results given in Table IV show that 6 pounds of washed Irish potatoes reduced the strength of the mercuric-chlorid solution to a slightly less extent than did an equal weight of sweet potatoes (Table II). They show further that Irish potatoes, like sweet potatoes, remove most of the mercuric chlorid during the first five minutes of treatment.

OTHER MATERIALS INVOLVED IN THE REMOVAL OF MERCURIC CHLORID

DIRT

For the purpose of determining the extent to which other materials associated with the treatment are responsible for taking up the mercuric chlorid from the solution, tests were made with dirt, sacks, a hamper, concrete, and a barrel. Three types of dirt were used. Sample 1 was composed of the sweepings from a sweet-potato storage house and consisted chiefly of soil and fine roots which had collected where crates had been moved about or potatoes picked over. This was run in duplicate. Sample 2 was obtained from a field on which rye had been grown during the previous autumn and spring at the United States Department of Agriculture Experimental Farm at Arlington, Va., and had just been plowed under a short time before the sample was taken. The soil was a dark loam and contained roots of rye and weeds. Sample 3 was a red clay subsoil almost or entirely devoid of humus, taken several feet below the surface. One pound of each sample was treated in 2 gallons of mercuric-chlorid solution, and the results are given in Table V.

TABLE V.—*Percentage of mercuric chlorid removed from solution by treating 1 pound of dirt in 2 gallons of solution*

Length of treatment.	Percentage of $HgCl_2$ present in solution.				Percentage of $HgCl_2$ removed from solutions.			
	Soil sample 1.		Soil sample 2.	Soil sample 3.	Soil sample 1.		Soil sample 2.	Soil sample 3.
Control [a]	91.1	100.0	101.9	94.0	.....	.....	.....	.....
5 minutes	75.8	82.4	100.5	93.0	15.3	17.6	1.4	1.0
10 minutes	75.5	82.0	97.6	92.2	15.6	18.0	4.3	1.8
30 minutes	75.5	82.0	.....	.....	15.6	18.0	.....	.....
60 minutes	75.8	.....	.....	.....	15.3	.....	.....	.....
24 hours	63.6	68.2	.....	.....	27.5	31.8	.....	.....

[a] Solution unused.

The results presented in Table V show a considerable difference in the amount of mercuric chlorid removed by the three types of soil. The two sets of soil sample 1 removed 15.3 and 17.6 per cent, respectively, during the first 5 minutes, after which there was little or no change up to and including 60 minutes. At the end of 24 hours there was a decided decrease in the strength of the solution. There was some variation in the changes produced by this dirt in the two trials, but in general the results are similar. However, a decided difference is apparent in the results of the experiment with soil sample 2. After 5 minutes only 1.4 per cent of the mercuric chlorid was removed, as compared with 15.3 per cent and 17.6 per cent with soil sample 1. Another increase to 4.3 per cent took place in the next 5 minutes, while in these cases the amount of mercuric chlorid seemed to remain constant for some time after the first 5 minutes.

Soil sample 3 behaved in much the same manner as sample 2, removing 1 per cent during the first 5 minutes and 1.8 per cent at the end of 10 minutes. It seems probable that the variation in the amount of vegetable matter present in the soils was largely responsible for the differences in the amount of mercuric chlorid removed, although other factors may have been involved. Soil adhering to the potatoes or that present in the containers is therefore responsible for the removal of a considerable part of the mercury. In view of these facts the extent to which a solution should be used depends somewhat upon the amount of soil and roots adhering to the potatoes.

SACKS

The amount of mercuric chlorid removed by two sacks treated in 2 gallons of solution was next determined. One was a 2-bushel cotton grain sack of the ordinary type, and the other a gunny sack of about equal capacity which had contained a commercial stock feed. The grain sack was washed and dried before being treated. The gunny sack was turned inside out and shaken thoroughly to free it as nearly as possible of adhering materials.

An examination of Table VI shows that a grain sack and a gunny sack remove a considerable amount of mercuric chlorid even during a 5-minute treatment and an increasingly larger amount as time goes on. If the rate at which a sack will remove the mercuric chlorid is proportional to the amount of solution in which it is treated, then the amount of mercuric chlorid removed by the bag from about 32 gallons would be something like 1 per cent for each treatment. Since the sacks continued to remove the mercury for at least 24 hours, they alone would be responsible for the removal of enough of the disinfectant to interfere seriously with the effectiveness of the subsequent treatments. It would therefore seem unsafe to use sacks as containers in the treatment of sweet potatoes.

TABLE VI.—*Percentage of mercuric chlorid removed from solution by treating sacks in 2 gallons of solution.*

Length of treatment.	Percentage of $HgCl_2$ present in solution.		Percentage of $HgCl_2$ removed from solution.	
	Grain sack.	Gunny sack.	Grain sack.	Gunny sack.
Control [a]	94.6	101.5	.....	.....
5 minutes	84.5	91.2	10.1	10.3
10 minutes	77.9	87.3	16.7	14.2
30 minutes	72.7	82.5	21.9	19.0
60 minutes	70.4	78.6	24.2	22.9
4 hours	65.4	.....	29.2	.....
5½ hours	.....	65.5	.....	36.0
24 hours	54.8	10.0	39.8	91.5

[a] Solution unused.

HAMPER

Sweet potatoes are often stored in bushel hampers, and it is sometimes convenient to use these as containers in making the treatments. Table VII gives the results obtained by treating a hamper in 7 gallons of solution contained in a 10-gallon stone jar.

TABLE VII.—*Percentage of mercuric chlorid removed from solution by treating a bushel hamper in 7 gallons of solution*

Length of treatment.	Percentage of $HgCl_2$ present in solution.	Percentage of $HgCl_2$ removed from solution.
Control [a]	97.3	.....
5 minutes	96.0	1.3
10 minutes	95.5	1.8
30 minutes	92.1	5.2
60 minutes	91.8	5.5
24 hours	68.8	28.5

[a] Solution unused.

An examination of Table VII shows that the hamper likewise removed some of the mercury, although a proportionally much smaller amount than the sacks. Nevertheless, it is to a certain extent responsible for reducing the strength of the mercuric-chlorid solution in the commercial treatments.

CONCRETE

Farmers sometimes use concrete tanks instead of barrels as containers for the disinfectant. In order to learn whether concrete will produce any change in the concentration of the mercuric-chlorid solution, the following experiment was conducted. Two concrete blocks, having a combined total area of 215 square inches and a weight of 9 pounds, with smooth surfaces except at one end, were immersed in 2 gallons of solution. The changes in the concentration of the solution are shown in Table VIII.

TABLE VIII.—*Percentage of mercuric chlorid removed from solution by treating concrete blocks in 2 gallons of solution*

Length of treatment.	Percentage of $HgCl_2$ present in solution.	Percentage of $HgCl_2$ removed from solution.
Control [a]	94.2	.....
5 minutes	94.0	0.2
10 minutes	94.0	.2
2 hours	90.7	3.5
24 hours	84.4	9.8

[a] Solution unused.

These figures show that practically no mercuric chlorid was removed from the solution during the first 10 minutes. However, in 2 hours the strength was reduced 3.5 per cent, and in 24 hours 9.8 per cent. These results demonstrate that although concrete does not remove the mercuric chlorid as rapidly as some of the other materials used, yet it does cause reduction in strength.

BARREL

Table IX gives the results of analyses of samples taken from a solution made up in a clean oak barrel. These results show that no appreciable loss of mercury could be detected up to the second hour, when 1.5 per cent had been removed. After 24 hours the strength of the solution was reduced 9.7 per cent. Obviously the barrel also exerts an influence upon the strength of the solution.

TABLE IX.—*Percentage of mercuric chlorid removed from solution by a clean oak barrel*

Length of treatment.	Percentage of $HgCl_2$ present in solution.	Percentage of $HgCl_2$ removed from solution.
Control [a]	100.1	.....
10 minutes	100.5	0
30 minutes	100.1	0
60 minutes	100.1	0
2 hours	98.6	1.5
24 hours	90.4	9.7

[a] Solution unused.

GENERAL DISCUSSION

From a survey of the tables it is evident that several factors may be responsible for the removal of mercuric chlorid from solutions used for treating sweet potatoes. No doubt the potatoes themselves remove a major portion of it, but other materials which come in contact with the solution, such as the sacks, hamper, soil, barrel, or concrete, also reduce the concentration of the solution. In the two experiments of which the results are recorded in Table I, the method of treating the sweet potatoes in common use among growers was used. Here potatoes, dirt, hamper, and barrel were all present, and the average amount of mercuric chlorid removed by them was approximately 1 per cent for each bushel treated. There was considerable variation in the amount removed by each 5 bushels treated, probably due at least in part to the amount of dirt clinging to the potatoes and to the differences in their size. By the addition of ¾ ounce of mercuric chlorid after each 10 bushels of potatoes treated the solution was kept near enough to its original strength for all practical purposes. However, ¾ ounce of mercuric chlorid in most cases was a little more than enough to restore the solution to its original

strength. Had the experiment been continued long enough it is possible that the strength of the solution would have been increased sufficiently to injure the potatoes, as sweet potatoes are very susceptible to mercuric-chlorid injury. It was found that 6 pounds of washed sweet potatoes removed slightly more than 1 per cent of the mercuric chlorid from 2 gallons of the solution. A pound of dirt in 2 gallons of solution removed from 1.8 to 18 per cent of the mercuric chlorid, the variation being due to the type of soil used. Aside from the potatoes, dirt is probably responsible for a greater change in the strength of the solution than any other single substance. However, sacks used as containers reduced the concentration very rapidly, a gunny and a grain sack in 2 gallons of solution decreasing the strength 16.7 and 14.2 per cent, respectively, in 10 minutes. On the other hand, a bushel hamper removed the mercury less rapidly. In view of these facts it would seem best whenever possible to use hampers or other wooden containers for the treatment. However, if it is necessary to use sacks the amount of mercuric chlorid added from time to time should be slightly increased. The oak barrel as well as the concrete blocks weakened the strength of the solution.

The foregoing facts show that the factors involved in disinfecting sweet potatoes are so many and varied that it is unwise to make any sweeping generalizations. However, if it is assumed that the average of approximately 5 per cent is removed by each 5 bushels of potatoes reasonably free from soil and treated in hampers in a clean barrel containing 32 gallons of solution, then the following recommendations may be made: After each 10 bushels of potatoes treated, add from $\frac{2}{5}$ to $\frac{1}{2}$ ounce of mercuric chlorid and add water to make the solution up to its original volume. It is further recommended that the solution be discarded after the treatment of 50 bushels.

Since the dirt and other foreign matter remove some of the mercuric chlorid, it is important that the barrel be thoroughly cleaned out before a fresh solution is made up. It is impossible to state that a given number of potatoes under known conditions will remove a definite amount of mercuric chlorid. The results of experiments 1 and 2 given in Table II illustrate this point, since in both cases 6 pounds of washed sweet potatoes of the same variety and from the same source were treated in 2 gallons of solution and in the former case 1 per cent and in the latter 2 per cent of the mercuric chlorid was removed in 1½ hours. No doubt the surface area of the potatoes governs to a certain extent the quantity removed. It is impossible to know the type or quantity of dirt and refuse that will find its way into the solution, and these are both important in reducing the concentration. All of these facts show how unreliable any sweeping generalizations may be. Nevertheless, there is need for some definite method of procedure in treating

sweet potatoes; and in view of the data presented in Table I, the writer feels that the recommendations given are more reliable than any heretofore proposed.

SUMMARY

(1) A bushel of sweet potatoes, when treated in 32 gallons of a 1 to 1,000 mercuric-chlorid solution in the manner generally followed by farmers, reduces the strength of the solution approximately 1 per cent.

(2) The decrease in the strength of the mercuric-chlorid solution is due in part to the potatoes themselves.

(3) The dirt and fibrous roots as well as the containers of both the potatoes and the solution also remove a certain amount of the disinfectant.

(4) Washed sweet potatoes and Irish potatoes remove approximately the same amount of mercuric chlorid from the solution.

(5) The addition of from $^2/_5$ to $^1/_2$ ounce of mercuric chlorid and sufficient water to make the solution up to its original volume after each 10 bushels of sweet potatoes treated will maintain the solution near enough to its original strength for all practical purposes for the treatment of 50 bushels of sweet potatoes.

CATALASE, HYDROGEN-ION CONCENTRATION, AND GROWTH IN THE POTATO WART DISEASE

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An attempt was made by one of the authors [1] to determine the relative importance of the physiological factors of the hydrogen-ion concentration, catalase, oxidase activity, and osmotic concentrations which are correlated with growth. Determinations were made upon tissues in which overgrowth was induced by inoculation with *Bacterium tumefaciens* Sm. and T. and as a result of injury from freezing.[2] In both of these cases the production of overgrowth was attended by an increase in catalase activity and decrease in hydrogen-ion concentration. It was shown to be improbable that overgrowths in tissues infected with *Bact. tumefaciens* were produced by differences in osmotic concentration brought about by the organism, since no considerable difference in osmotic concentration between diseased and healthy tissue could be found. In the mosaic disease of tobacco, in which a decreased growth is shown in the palisade cells of the diseased areas, there was a corresponding decrease in catalase accompanied by increased hydrogen-ion concentration of the expressed juice.[3] These cases would lead one to believe that increase or decrease in hydrogen-ion concentration brought about inverse changes in catalase, since it is known that catalase deteriorates rapidly in acid solutions and remains constant at acidities between P_H7 and P_H8. Boas [4] reports that the acidity of the cell sap of potatoes affected by leafroll is less than that of healthy plants; but whereas diseased plants show an increase in catalase content in some cases, this relation did not hold generally.

An idea of the importance of the factors correlated with the production of overgrowths can be obtained only by a study of conditions attending their production by various means or by different types of organisms. Excellent material for this work, in which there is a marked growth response on infection of the tissue, was found in the wart disease of the potato (*Solanum tuberosum L.*) casued by *Chrysophlyctis endobiotica* Schilb. These overgrowths are very striking in appearance and size,

[1] HARVEY, R. B. RELATION OF CATALASE, OXIDASE, AND H+ CONCENTRATION TO THE FORMATION OF OVERGROWTHS. *In* Amer. Jour. Bot., v. 7, no. 5, p. 211–213. 1920.

[2] ——— HARDENING PROCESS IN PLANTS AND DEVELOPMENTS FROM FROST INJURY. *In* Jour. Agr. Research, v. 15, no. 2, p. 83–112, 3 fig., pl. 7–11 and A (col.). 1918. Literature cited, p. 108–111.

[3] ——— HYDROGEN ION CHANGES IN THE MOSAIC DISEASE OF TOBACCO PLANTS AND THEIR RELATION TO CATALASE. *In* Jour. Biol. Chem., v. 42, no. 3, p. 397–400. 1920.

[4] BOAS, Friedrich. BEITRÄGE ZUR KENNTNIS DES KARTOFFELABBAUES. *In* Ztschr. Pflanzenkrank., Bd. 29, Heft 5/6, p. 171–176. 1919. Literaturverzeichnis, p. 176.

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frequently equaling or even exceeding the normal size of the organ which they replace, and they develop very rapidly—much more so than bacterial tumors. For instance, the earliest wart infections at Freeland, Pa., this year were found about July 13, and they were then not larger than 1 to 2 cm. in diameter. By the last of July warts 5 cm. or more in diameter were abundant, and some could be found even as much as 10 cm. in diameter and weighing 200 gm. Evidently growth is exceedingly rapid in the infected tissues, and this disease affords excellent material for a separation of the physiological factors affecting growth.

The hydrogen-ion determinations were made by the potentiometric method, using a bubbling electrode. The tests were run in pairs with wart and healthy tuber tissue collected from the same plant while in full vegetative activity and taken directly to the laboratory for testing. The material was ground in a food chopper and the juice pressed out through a cloth sack. No effort was made to use always uniform pressure with the different samples, because the hydrogen-ion concentration of an expressed juice depends not upon the total concentration of buffer substances present but upon the ratio of their concentrations. The samples were tested without dilution. It was observed that oxidase activity was much greater in wart tissue, judging by the very dark color which the expressed juice acquired as compared with the light color of healthy juice.

TABLE I.—*Hydrogen-ion concentration in healthy and wart tissue from the same plant*

Variety.	Healthy.		Wart.	
	P_H.	C_{H+}.	P_H.	C_{H+}.
Prince Henry	6.17	6.7×10^{-7}	5.96	1.09×10^{-6}
Ehnola	6.36	4.3×10^{-7}	Immune.	Immune.
Irish Cobbler	6.49	3.2×10^{-7}	Immune.	Immune.
Rural New Yorker	6.45	3.5×10^{-7}	6.11	7.76×10^{-7}
Do	6.39	4.07×10^{-7}	6.05	8.99×10^{-7}
Up-to-Date	6.35	4.47×10^{-7}	5.99	1.02×10^{-6}
Edzell Blue	6.59	3.16×10^{-7}	Immune.	Immune.
Seedling 39168	6.67	2.14×10^{-7}	Immune.	Immune.
Seedling 38816	6.65	2.24×10^{-7}	6.09	8.13×10^{-7}
McCormick	6.49	3.24×10^{-7}	Immune.	Immune.
Carman	6.57	2.69×10^{-7}	6.01	9.77×10^{-7}
Rose No. 4	6.48	3.31×10^{-7}	Immune.	Immune.
Portuguese Purple	6.65	2.34×10^{-7}	5.90	1.26×10^{-6}
Early Prospect	6.46	3.47×10^{-7}	6.03	9.33×10^{-7}
Average	6.49	3.48×10^{-7}	6.00	9.71×10^{-7}

The catalase determinations were made on diseased and healthy tissue from the same plant, using fresh material. The Van Slyke amino-nitrogen apparatus was used.[1] All determinations were made by using

[1] HARVEY, R. B. RELATION OF CATALASE, OXIDASE, AND H+ CONCENTRATION TO THE FORMATION OF OVERGROWTHS. *In* Amer. Jour. Bot., v. 7, no. 5, p. 211–213. 1920.

fresh 12-volume hydrogen peroxid (Dioxogen) previously neutralized with calcium carbonate $(CaCO)_3$. The amounts shown are cubic centimeters of O_2 evolved in 5 minutes of slow and approximately constant shaking. In most cases 50 gm. of tissue was ground with quartz and excess calcium carbonate in a mortar and made up to 1,000 cc. with water. In a few cases different amounts of tissue were used, but corrections have been made in the totals. The temperature during the tests could not be held constant, but the fluctuation was less than 2° C. for controls.

TABLE II.—*Catalase activity in healthy and wart potato tissue from the same plants as measured by cubic centimeters of oxygen evolved from 10 cubic centimeters of hydrogen peroxid in 5 minutes* [a]

Variety.	Temperature.	Healthy.		Wart.	
		Number of tests.	Average cubic centimeters O_2.	Average cubic centimeters O_2.	Number of tests.
	°C.				
Rural New Yorker	21	3	5.0	14.1	4
Do	21	3	5.9	.....	.....
Do	19 to 20	4	7.8	17.0	5
Do	20 to 21	4	9.2	17.9	9
Do	18.5	.....	.....	18.6	5
Do	19 to 20	.....	.....	22.4	7
Do	21.5	8	8.9	.....	.....
Do	20.5	4	5.0	.....	.....
Early Rose	21.0	1	7.4	.....	.....
Woodbury's White Rose	19.5	.....	.....	11.3	4
Do	23.5	4	10.4	.....	.....
Do	22.5	.....	.....	18.3	4
Prince Henry	19.2	.....	.....	11.7	5
Do	21.0	.....	.....	22.4	4
Do	22.2	4	6.4	.....	.....
Do	23.0	3	7.9	.....	.....
Portuguese Purple	22.5	.....	.....	28.1	4
Do	21.7	5	10.5	.....	.....
Average	.....	.....	7.8	17.9	.....

[a] Results of determinations for tubers of one plant are inclosed in braces.

The tables show the effect of this parasite in producing acidity in the tissues it infects, the average acidity of healthy tissues expressed by P_H 6.49, becoming P_H 6.00 in the warts. In no case does the acidity of healthy tissue approach that of diseased tissue. This indicates that the organism will be found to be an acid producer when it is successfully cultivated on artificial media and gives also the range of acidity for its activity in the host tissue. Similarly, catalase activity is very much greater in the overgrowths than in normal tubers, the values being expressed respectively by 17.9 and 7.8 cc. of O_2 evolved. In no instance does the catalase activity of healthy tubers approach that of warts by nearer than 10 per

cent, and in most cases is much less. The data therefore show conclusively that a strong positive correlation exists between catalase activity and growth even though the increased acidity of the medium would tend to increase the rate of destruction of catalase.

From the several studies here summarized, in which decreased acidity has been shown to be correlated with production of overgrowths and high catalase activity in two instances [1] and increased acidity with overgrowth production in the present case, it must be concluded that such changes in acidity have little to do with catalase or overgrowth, unless it should be found that increasing or decreasing the acidity toward a certain value lying about P_H 6.00 favors growth in the tissue affected. Acidity changes overlapping this value have not yet been found, but in the three cases investigated changes of reaction toward this value have been associated with overgrowth production. Further data are needed for deciding this point. It is remarkable how close the value for wart tissue lies to P_H 6.00. It varies not more than 0.1 P_H from this value, although the acidities of different potato varieties vary as much as 0.5 P_H from each other.

It is evident from Table I that differences in the acidity of the potato varieties are not associated with resistance to the disease as shown by the production of overgrowths.

SUMMARY

A study was made of hydrogen-ion concentration and catalase activity in a new type of plant overgrowth, the wart disease of the Irish potato caused by *Chrysophlyctis endobiotica*.

The hydrogen-ion concentration of wart tissue is constantly greater than that of healthy tubers from the same plant, the values being represented by P_H 6.00 and P_H 6.49, respectively.

Catalase activity is much greater in the wart tissue, the values being represented by 17.9 cc. of O_2 for diseased and 7.8 cc. for healthy tissue.

Catalase activity is strongly correlated with growth in spite of the higher acidity of the proliferation. This differs from other types of plant overgrowths previously studied in which diminished acidity is correlated with increased catalase and growth activity.

Differences in acidity of the varieties are not associated with immunity to the disease.

[1] HARVEY, R. B. RELATION OF CATALASE, OXIDASE AND H+ CONCENTRATION TO THE FORMATION OF OVERGROWTHS. *In* Amer. Jour. Bot., v. 7, no. 5, p. 211–213. 1920.

——— HARDENING PROCESS IN PLANTS AND DEVELOPMENTS FROM FROST INJURY. *In* Jour. Agr. Research, v. 15, no. 2, p. 83–112, 3 fig., pl. 7–11 and A (col.). 1918. Literature cited, p. 108–111.

EFFECT OF CROWNGALL INOCULATIONS ON BRYOPHYLLUM

By ERWIN F. SMITH

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In November, 1919, Michael Levine published a paper [1] in which he called in question some of my recent work on crowngall,[2] maintaining as the result of his own experiments: (1) That the shoots found in leafy crowngalls *originate from the tumor tissue* rather than from groups of normal totipotent cells disrupted and set growing by the growth of the tumor as I have maintained; and (2) that if my theory of embryomas is correct any totipotent tissue should be set growing—for example, dormant buds—yet in *Bryophyllum calycinum* Salisb. the crowngall organism (*Bacterium tumefaciens* Sm. and T.) *has no stimulating effect on the formation of shoots, but rather an inhibiting effect.*

I had never tried *Bryophyllum calycinum* for crowngall inoculation until after the appearance of Mr. Levine's paper; then it seemed worth while to make some experiments with it. From statements in his paper I thought the plant might react differently from tobaccoes, geraniums, and other plants which had given me numerous crowngalls containing abortive roots or shoots, but I have not found it to do so. In fact, working on the same plant and using the same microorganism (the one I originally sent to his colleague, Dr. Isaac Levin) I have obtained results quite like those obtained on other plants, as may be seen from the illustrations accompanying this paper.

In passing, it may be said that removal of leaves from the parent plant before inoculation is not good technic because they would then have

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[1] LEVINE, Michael. STUDIES ON PLANT CANCERS. 1. THE MECHANISM OF THE FORMATION OF THE LEAFY CROWN GALL. *In* Bul. Torrey Bot. Club, v. 46, no. 11, p. 447–452, pl. 17–18. 1919. Abstract *in* Exp. Sta. Rec., v. 43, no. 3, p. 242. 1920. The summary and conclusions of Mr. Levine's paper are as follows:

"1. *Bacterium tumefaciens* inoculated by pricks of a delicate needle into the marginal notches of a leaf of *Bryophyllum calycinum*, where totipotent cells are present, results in the formation of a crowngall as readily as in other plants used for inoculation but without leafy shoots.

"2. Inoculation of *Bacterium tumefaciens* into the tissue of a leaf of *B. calycinum* in the vicinity of a small bud causes the formation of a gall and interferes with the normal development of the bud or leafy shoot.

"3. Inoculation of *Bacterium tumefaciens* into the midvein of a young or old leaf detached from or attached to the mother plant results in the development of a large gall without the development of leafy shoots.

"4. Inoculation of *Bacterium tumefaciens* into the growing region of the stem of a young plant causes the development of the ordinary crowngall with the occasional and subsequent development of a leafy shoot.

"5. *Bacterium tumefaciens* does not cause the formation of leafy shoots in *Bryophyllum calycinum* but rather inhibits and retards their normal development, when inoculated into the totipotent cells which appear at the notches of the leaf."

[2] SMITH, Erwin F. CROWNGALL STUDIES SHOWING CHANGES IN PLANT STRUCTURES DUE TO A CHANGED STIMULUS. Preliminary paper. *In* Jour. Agr. Research, v. 6, no. 4, p. 179–182, pl. 18–23. 1916.

——— EMBRYOMAS IN PLANTS (PRODUCED BY BACTERIAL INOCULATIONS). *In* Bul. Johns Hopkins Hosp., v. 28, no. 319, p. 277–294, 1 fig., pl. 26–53 on 14. 1917.

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little food and a meager water supply and hence be under the worst possible conditions for developing tumors and especially tumors containing shoots, and because owing to this procedure one would not then be able to distinguish between the specific crowngall stimulus and the general stimulus of separation, which in Bryophyllum sets all the leaf buds growing.

Mr. Levine's most serious criticism is the statement that the shoots in crowngall develop from tumor cells. According to my observations the crowngall stimulus does not create totipotent cells out of tumor cells, but only sets growing those totipotent cells which already exist in the invaded tissues. The tumor cell is a disoriented degenerate cell, given over to a hasty vegetative growth. It is not an embryo cell, and I know of no evidence going to show that it can develop subsequently into normal tissues, organs, or the whole plant; on the contrary, it tends steadily toward decay. Moreover, since the tissues are not killed, what becomes of the bud when inoculations are made in the leaf axil if the numerous shoots which appear in various parts of the subsequently developing tumor (as for example in Plates 102 and 103) are not growths from dislodged fragments of the bud? Furthermore, when a deep crowngall develops under the normal cortex, the cortex is lifted up and grows with the growth of the tumor without being actually a part of the tumor tissue. Certainly its cells have normal orientation, function normally, are no part of the malignant tissue and have not originated from it, although they are borne on it, as in Plate 101. See also Bulletin of the Johns Hopkins Hospital, September, 1917, figures 70, 71, 72 (okra tumors), and many other figures which I have published.

Concerning the tissue of vessels and mature cells contained in the substance of crowngalls, one may call it *stroma* if he likes. Every tumor has a stroma (supporting tissues), and it is not likely that the stroma in plant tumors would be exactly like that in animal tumors, although the amount of stroma is extremely variable in the latter. The crowngall stroma is organized along with the tumor and forms an intimate part of it, but I can not think that it is necessarily developed *out of infected cells.* We shall not know positively, perhaps, until we are able to stain the bacteria in situ. As in malignant animal tumors, the crowngall stroma appears to me to be a growth of normal tissues (vessels and connective cells) stimulated by the presence of the abnormal cells, just as the stimulated roots and shoots are outgrowths of normal cells; yet the latter, being no part of the actual tumor tissue, have normally arranged tissues whereas the tracheids of a crowngall, being a more intimate part of its structure (stroma), are often contorted into the most bizarre forms by the growth of the tumor. A crowngall in its growth often surrounds normal cells just as a cancer may or bears them on its surface. The number of vessels in a crowngall depends not on the activity of the tumor so much as on the nature of the tissue invaded—that is, a tumor

originating in some extremely vascular tissue is itself very vascular, much more so than one originating in a nonvascular tissue, although the latter always contains some vessels (tracheids) just as any wound repair tissue does.

To return now to Bryophyllum, when suitable inoculations are made under a dormant bud—that is, immediately under a petiole—the developing tumor not only stimulates the bud to develop into shoots, as may be seen in Plate 101, but sometimes even causes, like the disease in the peach tree called the peach yellows, a secondary set of branches to appear on such shoots, as may be seen under the arrows in Plate 101. Here the tumor-stimulated shoots are the only axillary shoots on the plant. Often the tumor tissue subsequently invades the swollen base of such a shoot.

On the contrary, when the inoculations are made directly into the leaf axil—that is, into the dormant bud and into tissues immediately surrounding it—centers of infection and of active growth begin around the needle pricks, and these disrupt the bud in various directions, widely separating its fragments, as may be seen in thin sections under the microscope; and subsequently these fragments are just as certain to feel the tumor stimulus and to develop as is the whole bud in the previous case, only, the food supply and water supply being divided and limited by distortion and rupture of the vascular bundles, the result will be the development in the axillary tumor of several to many stunted shoots or mere buds, rather than one or two strong shoots. This is illustrated in Plates 102 and 103, which are back and front views of the same inoculated plant.

This stimulus, moreover, contrary to Mr. Levine, not only causes axillary buds of Bryophyllum to germinate, but also leaf-notch buds. To have made his test of any value (conclusion 2), Mr. Levine should have inoculated leaves while still on the plant and not after removal, when most of the leaf-notch buds will grow indiscriminately whether inoculated or not. Also they should have been young leaves. When done in the right way, the buds, stimulated by crowngall, develop, and no others, as may be seen in Plate 104, where out of about 400 dormant buds situated on the margins of the 26 leaves only those on 2 inoculated leaves have developed into shoots; and even here, if I had made my inoculations *only on well-developed leaves* some or all of my results might have been negative, as happened on one of the lower leaves of this same plant. That here the growth of the dormant buds into shoots is attributable to the inoculations in their vicinity and to nothing else is plain, because leaf-notch buds do not develop at all on undisturbed young shoots. In old plants they grow freely out of the leaf notches of old leaves which are still green and very firmly attached to the stem—that is, not pathological—but the order of their development is different—that is, they first appear on the basal leaves and gradually extend to

those farther up. In Plate 104 they have appeared on two leaves in the middle of a young shoot (uppermost leaves at time of their inoculation) *and on these only*. Many controls, and also observations extending over a period of several years, teach me that this unusual development of the leaf buds on an otherwise undisturbed young plant can be attributed only to the stimulus of the neighboring marginal tumors; and, consequently, the response of totipotent cells in *Bryophyllum calycinum* is not different from that of similar cells in other plants.

There remains to consider what will happen when the infected needle is thrust directly into leaf-notch buds rather than into their vicinity. Mr. Levine says it results in the formation of a crowngall without leafy shoots (conclusions 1 and 5). Plates 105 to 108 are a sufficient reply to this, although they represent only a small part of the results obtained. Here single needle thrusts were made directly into dormant leaf-notch buds (toward the top of the plant), usually on one side of the leaf only;[1] and in nearly every case shoots as well as tumors have developed, and some of the small, slow-growing tumors bore several shoots and roots (35 roots in Pl. 108). I had many cases like this where shoots developed only from the inoculated notches, other buds on the same leaves remaining dormant. Shoots taken from single inoculated leaf notches a month earlier than Plate 105 (which is reduced) are shown enlarged five times on Plate 106. Here the small tumors at their base can be seen very distinctly. It will be observed also that the neighboring uninoculated notches are free from shoots. Later both the shoots and the tumors became larger. On the small stimulated shoot in at least two cases (Pl. 107) the buds in its lower leaf axils (under the arrows) also pushed just as in the shoots of Plate 101 and others in that series.

Most of the crowngalls produced on midribs of *Bryophyllum calycinum* are free from leafy shoots. They are free, however, not for the reasons assigned, but because totipotent cells are rather rare in the midribs of this plant, which has sent most of them to the edges of the leaf. Only rarely, therefore, might one expect the infected needle to reach a group of such cells. That crowngalls containing shoots and roots may be produced by midrib inoculations on Bryophyllum is sufficiently evident from Plates 109 and 110.

Totipotent cells occur abundantly in the midribs of tobacco, tomato, begonia, and pelargonium, but it does not follow that they occur in the same abundance in the midribs of all plants, and especially not in those of a plant as peculiar as this one. Where we *know* beyond peradventure

[1] This was probably not done in the best way to induce a breaking up of the bud tissue, since the needle was not thrust from the margins inward but from some distance inside the margin outward through the leaf parenchyma, parallel to the leaf surface, until it was judged that the bud had been reached, a matter always in some doubt because of its invisibility and small size. Three distinct shoots are the most I have seen develop from such an inoculation, but undoubtedly the bud can be ruptured so as to produce a much larger number of shoots.

that there are totipotent cells—in the leaf axils and in the leaf notches of Bryophyllum—*there I have got the same results with crowngalls as on other plants.*

I undertook to test my conclusion as to the rarity of totipotent cells in the midrib of *Bryophyllum calycinum* in another way—that is, on 150 well-developed leaves by means of sand-bed experiments. The margins were trimmed away and after some days the leaves were removed from the plants and bedded on damp sand, with the undersurface down and petiole buried. Just before this was done the midribs were cut crosswise in from 4 to 12 places, depending on the size of the leaf. After six weeks they were removed and examined. Of these leaves, 27 per cent developed roots (and sometimes shoots) from the base of the petiole, showing that totipotent cells are of fairly frequent occurrence in that organ, and 10 shoots appeared on the margin of leaf blades in spite of the fact that they had been trimmed, but, with exception of two cuts on one leaf where incipient roots appeared, none whatever (either roots or shoots) appeared on the cut midribs, although the conditions were favorable and there were at least 1,000 opportunities. This tends to confirm the conclusions derived from the crowngall inoculations. I conclude, therefore, that shoots are rare in the midrib tumors for the sole reason that totipotent cells are rare in such places. Yet if organs are developed directly from tumor cells, as Mr. Levine maintains, then why in the presence of such an abundance of tumor cells in active growth are shoots and roots so rare in midrib tumors on Bryophyllum? These tumors grow as vigorously as axillary tumors on Bryophyllum or as midrib tumors on tobacco, and should we not expect to find shoots as common in the former as in the latter, if this hypothesis is correct?

The exact nature of the stimulus that sets the leaf-notch buds growing in crowngall inoculation may be left for discussion elsewhere. It is sufficient here to have established that, contrary to Mr. Levine's statements, a distinct crowngall stimulus exists for the dormant buds of Bryophyllum.

PLATE 101

Middle part of shoot of *Bryophyllum calycinum*, showing results of inoculation with *Bacterium tumefaciens* (hop strain) on March 10, 1920. The needle was set in horizontally just below the lower left petiole. As the tumor developed it twisted the leaf and shoot and caused the bud above it to grow. This axil was the only one on the plant that developed any shoots. Other plants not photographed behaved in the same way. The shoots show secondary branching (under the arrows) and enormous enlargement at the base. At *XX* leaves were broken off. Root anlage at *R*. Photographed June 17, 1920.

R

PLATE 102

Crowngall embryoma on *Bryophyllum calycinum*. The hop strain of *Bacterium tumefaciens* was inoculated in upper leaf axils January 13, 1920, from a 5-day agar streak by needle pricks. Many of the tumor shoots were red or purplish red. Photographed June 2, 1920. See Dr. Levine's conclusion No. 4.

PLATE 103

Same plant as in Plate 102, but showing the other side of the tumors. Tumors with shoots in three-leaf axils. Equally striking results were obtained on another plant.

PLATE 104

Young shoot of *Bryophyllum calycinum*, showing crowngall stimulus—that is, a pair of leaves sending out shoots from their margins as a result of inoculating *Bacterium tumefaciens* (hop strain) in the vicinity of the leaf notches. This pair was plainly dwarfed. Inoculated January 13, 1920. Actual height of shoot 24 inches. Some of the upper leaflets were knocked off in bringing the plant from the hothouse to the laboratory to be photographed, but none of these bore any shoots. There were 12 shoots on the inoculated leaves. Photographed June 7, 1920.

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PLATE 105

Further evidence that crowngall inoculation stimulates bud development. Pairs of leaves from two plants inoculated June 23, 1920, and photographed August 31. Nearly every inoculated leaf notch has given rise to a shoot. No shoots from the lower (uninoculated) leaves or from uninoculated notches on the upper leaves. From *B* at *X* proliferated notches were cut away at the end of July for some of the photographs of that date (Pl 106). These inoculations were made by single needle thrusts directly into the region of the bud. A, natural size; B, about one-third natural size.

A
X
B

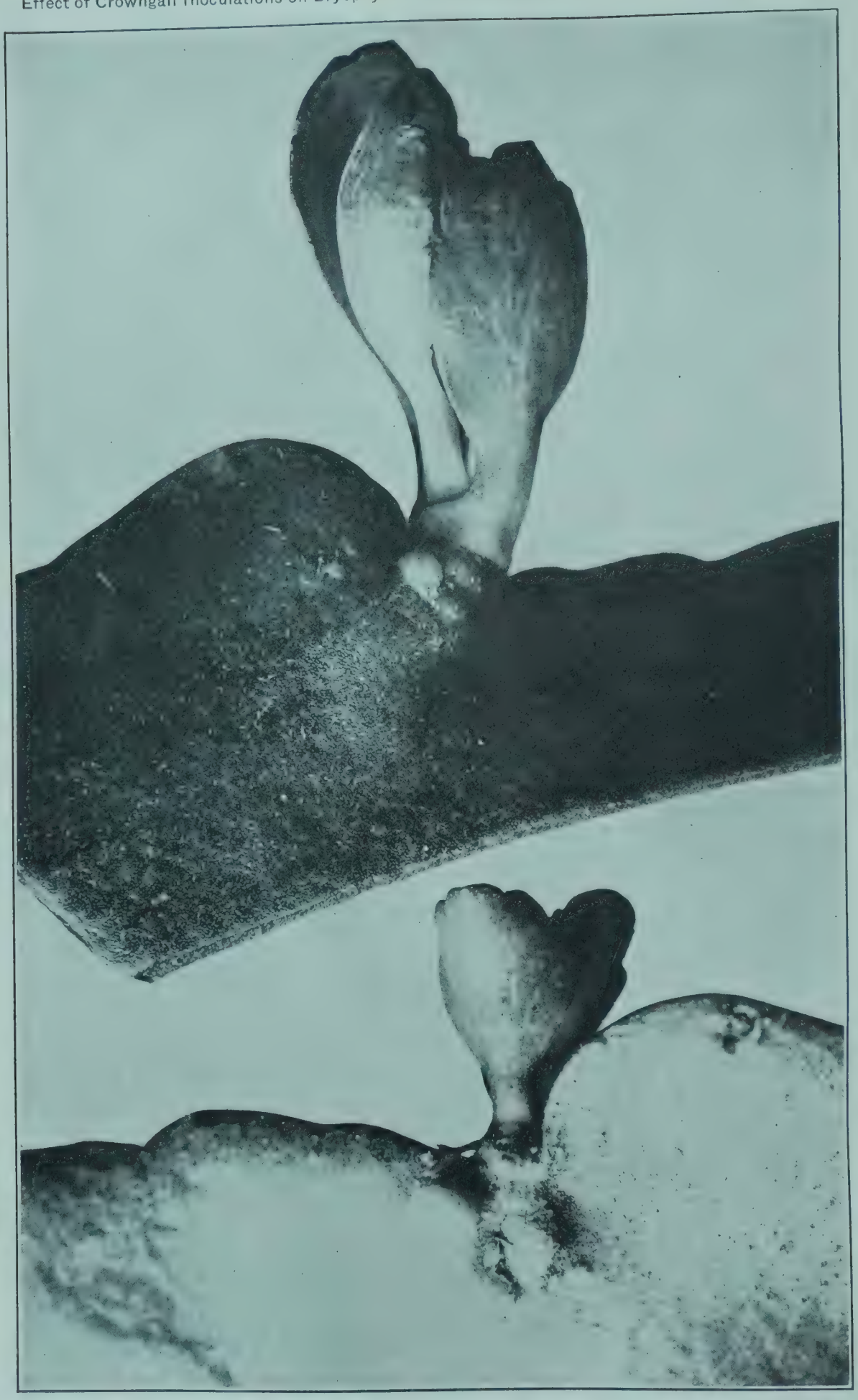
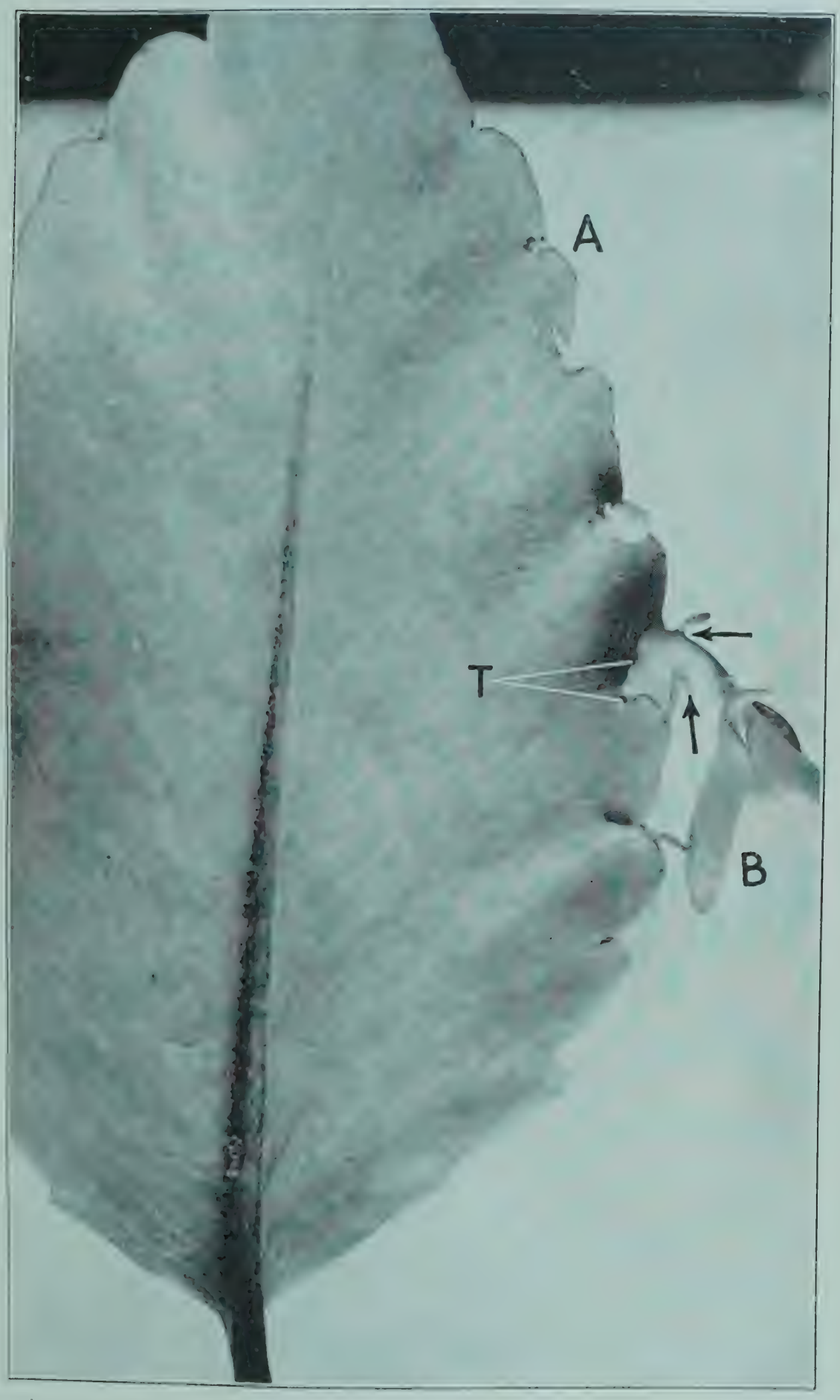


PLATE 106

Inoculated leaf notches cut away and photographed July 27, 1920, and enlarged to show the small tumors distinctly. Each was produced by a single needle prick introducing *Bacterium tumefaciens* (hop strain). Such shoots developed about a month after the inoculation and only from the inoculated notches. Most bore roots also, as in the lower shoot. × 5.

PLATE 107

Same series as Plate 108 but collected a month later. Only the five middle notches from *A* to *B* were inoculated. The arrows point to buds germinating in the lower leaf axils. The shoot is pushed out of its original location and is now borne on the top of the growing tumor, which, owing to the method of inoculation already described, began underneath it. The tumor proper lies at the base of the triangle *T*. Immediately above this is the swollen base of the shoot normal on its surface (structurally) but diseased within—that is, containing groups of disoriented tumor cells. Inoculated June 11, 1920. Photographed October 20. Natural size.


A
T
B

R
Rs
X

PLATE 108

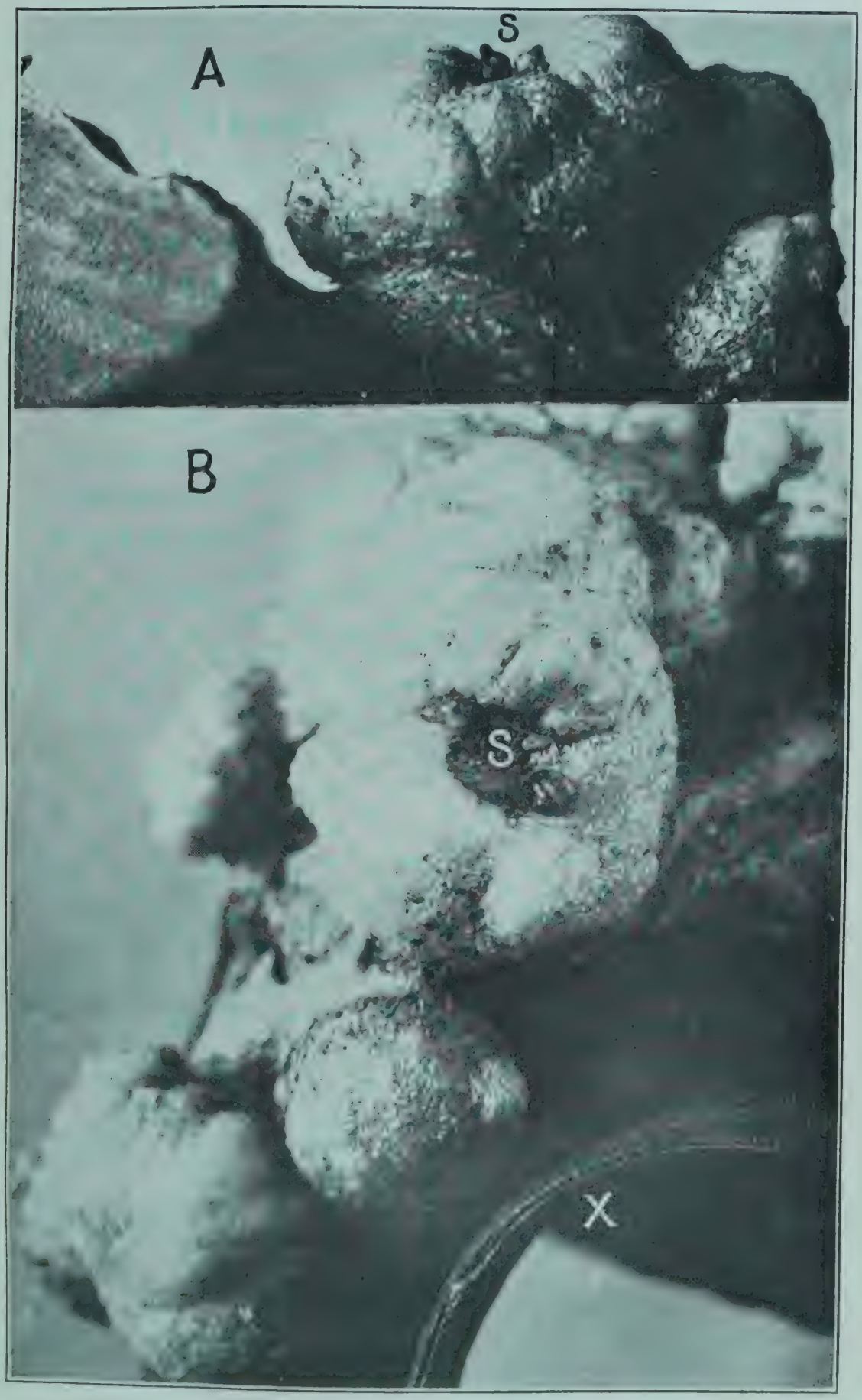
Crowngall from a leaf notch of *Bryophyllum calycinum*, showing 3 shoots and 35 roots. The arrow indicates the dividing line between naked tumor tissue (below) and the stretched, lifted up cortex (above) which contains normally oriented cells and surrounds disoriented tumor cells. *Rs*, roots; *R*, pushed up cortex where a root originating in the pericycle of the larger shoot has not yet reached the surface. At *X* another leaf notch was inoculated, but the tumor tissue (shown to be present by means of serial sections) is quiescent and the shoot is barely visible; yet judging from results obtained on the orange,[1] it might later have developed into a tumor as large as the upper one, especially if that had been removed. Inoculated June 11, 1920. Photographed September 22. ×5.

[1] SMITH, Erwin F. AN INTRODUCTION TO BACTERIAL DISEASES OF PLANTS. Fig. 342. Philadelphia and London. 1920.

PLATE 109

A.—Side view of a pure white midrib tumor on *Bryophyllum calycinum*, showing at *S* two pale green shoots.

B.—Vertical view of A, showing the green shoots at either side of *S* and at *X* the twisted leaf blade in cross section. The leaf was bent double by growth of the tumor. × 5.


A
S
B
S
X

X
M

PLATE 110

Midrib crowngall on *Bryophyllum calycinum*, showing 5 roots developing from one of the tumor lobes at *X*. There were also 16 roots developing from other lobes under the arrow. Midrib at *M*. Midrib tumors on three other leaves in this series also showed roots. Inoculated June 23, 1920. Photographed November 18. × 5.

EFFECT OF THE LENGTH OF DAY ON SEEDLINGS OF ALFALFA VARIETIES AND THE POSSIBILITY OF UTILIZING THIS AS A PRACTICAL MEANS OF IDENTIFICATION

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At Arlington Farm, Va., a few years ago the writers noted striking differences in the habits of growth of young seedlings of the commercial varieties of alfalfa produced from seed sown in the early fall. The differences in height and general appearance were so great that even a casual observer could easily distinguish certain of the varieties. Not having noted previously such a marked varietal difference in seedlings, the writers were led to the tentative conclusion that the time of sowing was, in some measure at least, responsible for the phenomenon. To test the validity of this conclusion, experiments were conducted the following year in which Peruvian,[1] Kansas,[2] Canadian variegated, Grimm, and a yellow-flowered alfalfa (*Medicago falcata* L.) were sown at Arlington Farm in the early fall. The same striking differences that had previously been observed were noted. However, May and June sowings at Arlington and also at North Ridgeville, Ohio,[3] showed no such marked varietal characteristics. This, in a measure, confirmed the conclusions the writers had reached and suggested further investigations, which were subsequently conducted.

POSSIBILITY OF DISTINGUISHING VARIETIES OF ALFALFA IN THE SEEDLING STAGE[4]

In addition to studying the effect on alfalfa seedlings of sowings made at different times of the year, it occurred to the writers that it might be possible in this connection to develop a practical method of identifying various alfalfas in a relatively short time after samples of seed are available. Such a method would be useful, indeed, especially in identifying the Grimm and Peruvian varieties. In fact, it was chiefly to find a means of identifying alfalfas quickly that detailed studies of seedlings were undertaken.

Partly because of availability of location and partly for the purpose of testing the effect of latitude on the growth of alfalfa seedlings,

[1] All references to Peruvian alfalfa in this publication are to the hairy strain.

[2] For the sake of convenience the term Kansas alfalfa refers to the regional strain of common alfalfa produced in Kansas.

[3] Experiments conducted cooperatively with the Ohio Agricultural Experiment Station at North Ridgeville, Ohio, about 3 miles north of Elyria.

[4] For the sake of convenience the term variety is used in its broad sense to mean regional strains as well as distinct horticultural varieties.

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sowings were made at North Ridgeville, Arlington Farm, and Bard, Calif. Early September sowings gave practically the same results at North Ridgeville as at Arlington Farm. The late fall and early winter sowings at Bard produced seedlings of much the same appearance as those from early September sowings at North Ridgeville and Arlington. This was also true of December sowings in the greenhouse at Washington. May and June sowings at Arlington and North Ridgeville showed practically no difference in the rapidity and habit of growth of the varieties tested. A range of field sowings from August 26 to September 16 at Arlington showed that five to six weeks of growing weather at that time of the year are required to produce plants of a satisfactory size for studying the varietal characters of the seedlings. The late August and early September sowings gave good results, but September 16, in this particular case, proved to be rather late.

From these tests it was evident that by sowing seed in late summer or early fall in the latitude of Arlington, and northward, it is easily possible to determine whether a given lot of alfalfa is of the Peruvian variety, the common variety (such as the various regional strains) or the variegated (such as the Grimm, Baltic, and Canadian variegated). From spring or summer sowings, especially in the North, the varietal differences exhibited are hardly great enough to be regarded as sufficient for the untrained person to recognize with certainty. Thus far in the investigations this method is limited in its application to field sowings in the fall in the North and to winter sowings in the extreme South. It is also limited to distinguishing between groups of alfalfas, such as the common or the variegated, rather than between the strains in these groups, as for example the Kansas-grown common strain and the Dakota-grown common strain or the Grimm, Baltic, and Canadian variegated. But the method makes it easily possible to distinguish between the Peruvian and the other alfalfas.

COMPARATIVE CHARACTERISTICS OF SEEDLINGS

The differences that were most noticeable in the seedlings resulting from fall sowings at Arlington were those of height, branching, and compactness. The Peruvian produced erect, sparsely branched seedlings of greater height than those of the other varieties. They were also more nearly uniform. The seedlings of the Kansas variety very closely resembled those of the Peruvian variety in uniformity and general habit of growth but were not nearly as tall and showed more of a tendency to send out basal branches. The Grimm seedlings were much shorter, more branched, and less erect than those of the Peruvian or Kansas alfalfas. They also showed more variation than these varieties. The seedlings of *Medicago falcata* were uniform, very short, and exhibited a rosette-like habit. The Grimm seedlings were nearly intermediate between the Kansas and *M. falcata*. The difference between the seedlings

of the several varieties is well illustrated in Plates 111 and 112. While there were some differences between the seedlings of the several varieties from May and June sowings, the Kansas and Grimm more nearly approached those of the Peruvian in height and very closely resembled each other. The Grimm seedlings did not take on the branching or rosette-like habit of growth so characteristic of seedlings from fall sowings. The spring seedlings of the Grimm were also much more uniform than the fall seedlings and closely resembled the Kansas and Peruvian in this characteristic. (Pl. 113, 114, 115.)

RELATION OF ALFALFA SEEDLINGS TO PHOTOPERIODISM

After a study of the behavior of the alfalfa seedlings from sowings made at different times of the year and at different latitudes it was concluded that the marked differences exhibited by seedlings of the several varieties from fall sowings at Arlington and North Ridgeville were due chiefly to the effect of cool weather. With no marked varietal differences from the May and June sowings, this conclusion seemed entirely justified. The significance of the fact that greenhouse tests made in December at Washington produced striking differences between the varieties was not appreciated by the writers at that time. Later, however, the results of Garner and Allard [1] from their investigations in the photoperiodism of plants caused the writers to suspect that the length of day might, to some extent, be involved in the phenomenon exhibited by the seedlings of the various alfalfas included in their studies. Consequently experiments were outlined and undertaken in the greenhouse on the Department of Agriculture grounds at Washington. On January 20 seed of Peruvian alfalfa, Kansas, Grimm, Turkestan, and a strain of *Medicago falcata* was sown in three flats. One flat was given the effect of the normal length of day, one was put in total darkness at 4 p. m. and left there until 9 a. m., and one had the length of day augmented by an electric light and reflector placed about 2 feet above it. The light was turned on a half hour before sundown each day and turned off at 11 p. m. The length of both the short and long day was fixed at what was regarded as extreme. The former was fixed largely as a matter of convenience to suit the working hours of the gardener and the latter to insure the effect of a considerably increased period of illumination. The day-night treatments apparently produced no effect on the germination of the seeds, but as is almost invariably the case the *M. falcata* seed germinated more slowly and unevenly than the others. The Grimm and Turkestan resembled the *M. falcata* in a general way in these respects. The Peruvian and Kansas seed germinated with about the same rapidity and uniformity. The differences, if any, were in

[1] GARNER, W. W., and ALLARD, H. A. EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. *In* Jour Agr. Research, v. 18, no. 11, p. 553-606, 3 fig., pl. 64-79. 1920. Literature cited, p. 605-606.

favor of the Peruvian. Ten days after the seedlings emerged some differences were evident. These differences increased rapidly, and on March 3 typical plants from each series were removed to the laboratory and photographed. The photographs tell the story. (Pl. 116–121, A.) The seedlings given the normal day which, at this time, was of about the same length as the average October day, behaved in practically the same manner as those grown in the greenhouse from December sowings and the same as those produced from early fall field sowings at Arlington. The seedlings under the light of the short day—from 9 a. m. to 4 p. m.—exhibited the same varietal differences as those under the normal day; but the differences were accentuated, as the photographs indicate. The seedlings grown under the long day—under the day with added illumination from twilight until 11 p. m.—behaved quite differently from the others. The seedlings of *M. falcata* instead of being short and rosettelike in habit, grew erectly with no branching and attained practically as great a height as any of the seedlings in the three series, up to the time photographed. Later on, however, these seedlings were overtaken by the other varieties. The Grimm and Turkestan behaved similarly to *M. falcata;* and, whereas the Peruvian and Kansas seedlings were erect, they failed to keep the relative position with regard to height which they held under the normal and short day. In brief, the abnormally long day produced almost a reversal of order in the matter of height and to a degree a reversal in the habit of growth. The temperature of the greenhouse in which the tests were conducted ranged from 50° to 60° F.; and while the electric light under which the long-day seedlings were placed produced a slight rise of temperature, this did not quite equal the temperature inside of the dark chamber and is considered insignificant so far as its effect on the general results is concerned. The dark chamber was a wooden box 3 by 3 by 3 feet, of ⅜-inch material with light-tight ventilators, top and bottom. (Pl. 121, B.) With the factor of temperature virtually eliminated, it seems fair to conclude that the phenomenon of difference in habit of growth of seedlings of alfalfa varieties obtained both in the field and greenhouse experiments herein described was due, for the most part, to the day-night relation of the varieties.

REACTION OF VARIETIES AND SPECIES TO LENGTH OF DAY IN ACCORDANCE WITH REGIONAL DEVELOPMENT

The evidence is quite clear that Peruvian alfalfa grows best in comparison with others under the relatively short day while the strain of *Medicago falcata* used in these experiments produces its best growth under the relatively long day. It also seems to be true that the Kansas strain resembles the Peruvian in its day-night relations while the Grimm resembles the *M. falcata.* This is not surprising. The Peruvian variety was developed near the Equator where the days are always about 12 hours long. The Kansas, which is the result of several seed generations

grown in that State from original stock probably produced in southern latitudes, must therefore be regarded as a southern strain. The *M. falcata* was from the original stock secured by the Department of Agriculture from eastern Russia. The Grimm strain is a hybrid between *M. sativa* L. and *M. falcata* that has been locally grown in northern latitudes for more than half a century. The Turkestan used in this case behaved much the same as the Grimm; but other lots will doubtless show considerable variation in this respect because of the great range in comparative length of day in different parts of Turkestan. The relation between locality of origin of the various alfalfas and their reaction to the day-night period is shown consistently.

The day-night experiments herein described throw light on some questions that have perplexed students of alfalfa in this country. It is well known that the Peruvian variety in the Southwest continues growth later in the fall than the other commercial varieties and begins growth earlier in the spring. This fact led Brand [1] to conclude that it was able to grow at a lower temperature than the other varieties. He applied the term "zero point" to the minimum temperature at which growth takes place and classed Peruvian alfalfa as a variety with a low zero point. From subsequent investigations it was found that while Peruvian alfalfa continues growth late in the fall and begins it early in the spring in the Southwest and even as far north as Washington, D. C., it does not do so at Redfield, S. Dak., and other northern points. Oakley and Garver [2] were of the opinion that this phenomenon was due to the effect of very low temperature to which the variety was subjected in the North. It is quite clear now that this explanation is not the proper one, for although temperature bears an important relation to growth, it is its day-night relation that causes Peruvian alfalfa to have a longer growing period than the other varieties in the South and to have the same or even a shorter growing period in the North. The northern varieties do not thrive under the short day of southern Arizona and California, but they can more than compete with the Peruvian under the long day of the North. At Redfield the growing season does not extend far into the short days of late fall or winter, neither does it begin in the spring until the long days have arrived. Low temperature in the fall checks the growth of Peruvian alfalfa in the North before the short days give it the advantage over *Medicago falcata*, Grimm, and other northern alfalfas. Low temperature also holds the Peruvian back in the spring until the long days arrive under which the northern alfalfas grow most rapidly. An explanation is now found for the fact that while Grimm alfalfa gives better yields than the Kansas in the North it does not do so as far south

[1] BRAND, Charles J. PERUVIAN ALFALFA: A NEW LONG-SEASON VARIETY FOR THE SOUTHWEST. U. S. Dept. Agr. Bur. Plant Indus. Bul. 118, 35 p., 11 fig., 3 pl. 1907.

[2] OAKLEY, R. A., and GARVER, Samuel. MEDICAGO FALCATA, A YELLOW-FLOWERED ALFALFA. U. S. Dept. Agr. Bul. 428, 70 p., 23 fig., 4 pl. 1917. Literature cited, p. 67–70.

as Virginia. It is an easy matter now to account for the fact that certain lots of imported Turkestan alfalfa give comparatively high yields at Redfield and other northern points while the yields are comparatively low in Virginia and other southern States.

As a result of Garner and Allard's[1] discovery of the photoperiodism of plants it appears necessary to change the recommendations commonly made in regard to the range and adaptation of alfalfa varieties. Instead of recommending the use of southern varieties, such as the Peruvian and Kansas, as far north as they are hardy, these varieties should be recommended for as far north as the length of day during the growing season gives them an advantage over the northern alfalfas that yield comparatively better under the relatively long day. There is a difference between the two types of recommendation, especially along the Washington and Oregon coast and in such areas as the Judith Basin, Mont., where the winters are not so severe as at some other points of the same latitude. Peruvian alfalfa as a rule does not winterkill in the former region, and the Kansas seldom winterkills in the latter. Both sections have a length of day during the growing season that favors the Grimm and its allies. As a matter of fact, the Grimm has given somewhat better yields than the Kansas at Moccasin, Mont. The day-night relation of alfalfa is a much better measure of the adaptability of the varieties than their ability to endure cold and unfavorable winter conditions generally, as is indicated by the fact that Kansas alfalfa is sometimes hardy in the North where the length of the day is less favorable to it than to the Grimm and other northern varieties.

It is believed that by the methods followed by the writers in their recent experiments it will be quickly and easily possible to tell within reasonable limits from a given lot of alfalfa seed in what sections it will give the best yields of hay as compared with other varieties. It may even be possible by this method to determine in a measure to what extent it is winter hardy. Investigations are now being made in this field, and it is confidently expected that a careful study of the day-night relations of alfalfa will throw considerable light on the subject of the development of regional strains.

HOW ALFALFAS MAY BE IDENTIFIED BY SEEDLING CHARACTERS

It is exceedingly difficult if not impossible to identify varieties of alfalfa by seed characters. Where identification is important, as it is in many cases, any short-time method that will assist in accomplishing it is useful. It is generally possible to distinguish between imported and domestic seed by the weed seeds and other incidental impurities contained in the sample. This is particularly true of imported seed of Turkestan alfalfa, but it is practically impossible to distinguish seed of

[1] GARNER, W. W., and ALLARD, H. A. OP. CIT.

Grimm alfalfa from that of other domestic varieties. Analysts, it is true, become very expert in identifying kinds of alfalfa seed by various pieces of evidence, including weed seeds, dust, and all incidental impurities, as well as by color, size, and percentage of hard seed. But even seed analysts can not always make the critical determinations that are necessary. To the less well-trained person the seed of all varieties of alfalfa is very much alike.

By sowing seed in the greenhouse, as was done in the experiment herein described, it is possible within three or four weeks to distinguish seedlings of one kind from another. If this method is used, it is highly desirable to have on hand viable seed of the most important commercial alfalfas and of at least one strain of *Medicago falcata* to be used in making the necessary comparisons of the seedlings. As a simple method to follow, it is suggested that the seed of the lot or lots to be identified be sown in rows in small flats in which adjoining rows are sown with seed of the Peruvian, Kansas, or some southern-grown common strain, Montana, Dakota, or some northern-grown common strain, Grimm, and a strain of *M. falcata*. For best results there should be three flats, each sown in the same way—one flat to be subjected to a short day, one to an abnormally long day, and one to the normal day as it exists at the time the tests are made. The normal day series may not be necessary, but there are some advantages in including it as a control. It is suggested that the day be shortened for the first series by putting the flat in a room with light excluded or in a box such as is shown in Plate 121, B, at 4 p. m., and leaving it in total darkness until 9 a. m. This gives a sufficiently long day for the necessary photosynthetic action and at the same time it is short enough to produce quick and striking differences in the seedlings of at least certain of the varieties. The length of day may be augmented for the second series by using a tungsten bulb of 100 watts and a reflector as shown in Plate 121, B. It is suggested that the light be turned on at dusk and turned off about 11 o'clock p. m. This gives an abnormally long period of constant illumination, but such a period is helpful in producing striking results quickly. By the use of both the short and the long day the differences between the northern and southern alfalfas are better shown. The normal-day series adds evidence when the differences of the seedlings are studied.

While it is not possible by this method to distinguish between certain closely related varieties or strains such as the Grimm, Baltic, and Canadian variegated, these strains as a group can be positively distinguished from the common and other commercial strains by the general habit of growth and lack of uniformity of the seedlings. While the Turkestan seedlings can easily be distinguished from the Kansas by their height and lack of uniformity, they resemble those of the Grimm closely enough to make positive identification difficult. Since there is great diversity in the various lots of Turkestan seed that are imported commercially,

it is probable that weed seed and other impurities in the seed will be more satisfactory than the seedling characters as a means of identification. The regional strains of common alfalfa may be distinguished from the Peruvian, on the one hand, by their more rapid very early growth under the long day and from the Grimm, Baltic, Canadian variegated, and Turkestan, on the other, by their greater height, erectness, and uniformity under the short day. Table I sets forth briefly the characteristics of the seedlings of the various alfalfas under the different lengths of day as determined by experiments conducted in the greenhouse at Washington, D. C.

TABLE I.—*Characteristics of seedlings exposed to varying periods of light daily. Sown in greenhouse January 20; notes taken on March 3, 1921*

Varieties.	Exposed to normal day with electric light from dusk to 11 p. m.	Exposed to normal day.	Exposed to daylight from 9 a. m. to 4 p. m.
Peruvian	Erect, no branching, height 5 inches.	Erect, no branching, height 3⅔ inches.	Erect, no branching, height 3 inches.
Kansas[a]	Erect, no branching, height 4½ inches.	Erect, no branching, height 3½ inches.	Erect, no branching, height 2½ inches.
Grimm	Erect, no branching, height $4^4/_5$ inches.	Low and spreading, stipules enlarged, basal branches just showing, height 2½ inches.	Low and spreading, rosette-like, stipules enlarged, basal branches just showing, height 2 inches.
Turkestan	Erect, no branching, height $4^4/_5$ inches.	Low and spreading, stipules enlarged, basal shoots just showing, height 2 inches.	Low and spreading, rosette-like, stipules conspicuous, basal shoots just showing, height 1⅔ inches.
Medicago falcata	Erect, slender, no branching, height 5 inches.	Low and rosette-like, stipules conspicuous, height 1¼ inches.	Low and rosette-like, stipules conspicuous. height 1 inch.

[a] These measurements were taken too late to show the proper relation between seedlings of the Kansas and those of the Peruvian.

By the use of this method and by the aid of such facts as can frequently be had regarding the lots of seed to be identified, it will be found possible in many cases to make a reasonably certain varietal identification. The method is offered especially to Experiment Station workers and those engaged in the administration of alfalfa seed certification activities as an aid to and not as a positive means of identifying varieties. It is recognized that the work herein reported upon is far from complete, but investigations are now being conducted to make it more comprehensive and useful.

The method involving the sowing of seed in the field is not recommended where a greenhouse is available. It is less certain and its use is limited to sections where the short days of the year are sufficiently warm to produce growth.

SUMMARY

When seed of Peruvian, Kansas-grown common, Grimm, and Turkestan alfalfa are sown in the early fall in the approximate latitude of Washington, D. C., the seedlings develop characteristics that make it easily possible for the casual observer to distinguish one variety from another. Seedlings from spring sowings did not develop differences at Washington or in northern latitudes in the United States that would be apparent to the untrained eye. Seedlings from greenhouse sowings in December at Washington showed essentially the same distinguishing characters that were shown by early fall sowings in the field at Arlington.

At first it was concluded that the temperature of the fall days was responsible for the striking differences shown by the alfalfa seedlings. However, the results of Garner and Allard's work on the effect of the length of the day on the growth of plants suggested the advisability of growing alfalfa seedlings in the greenhouse under artificially lengthened and also under artificially shortened days.

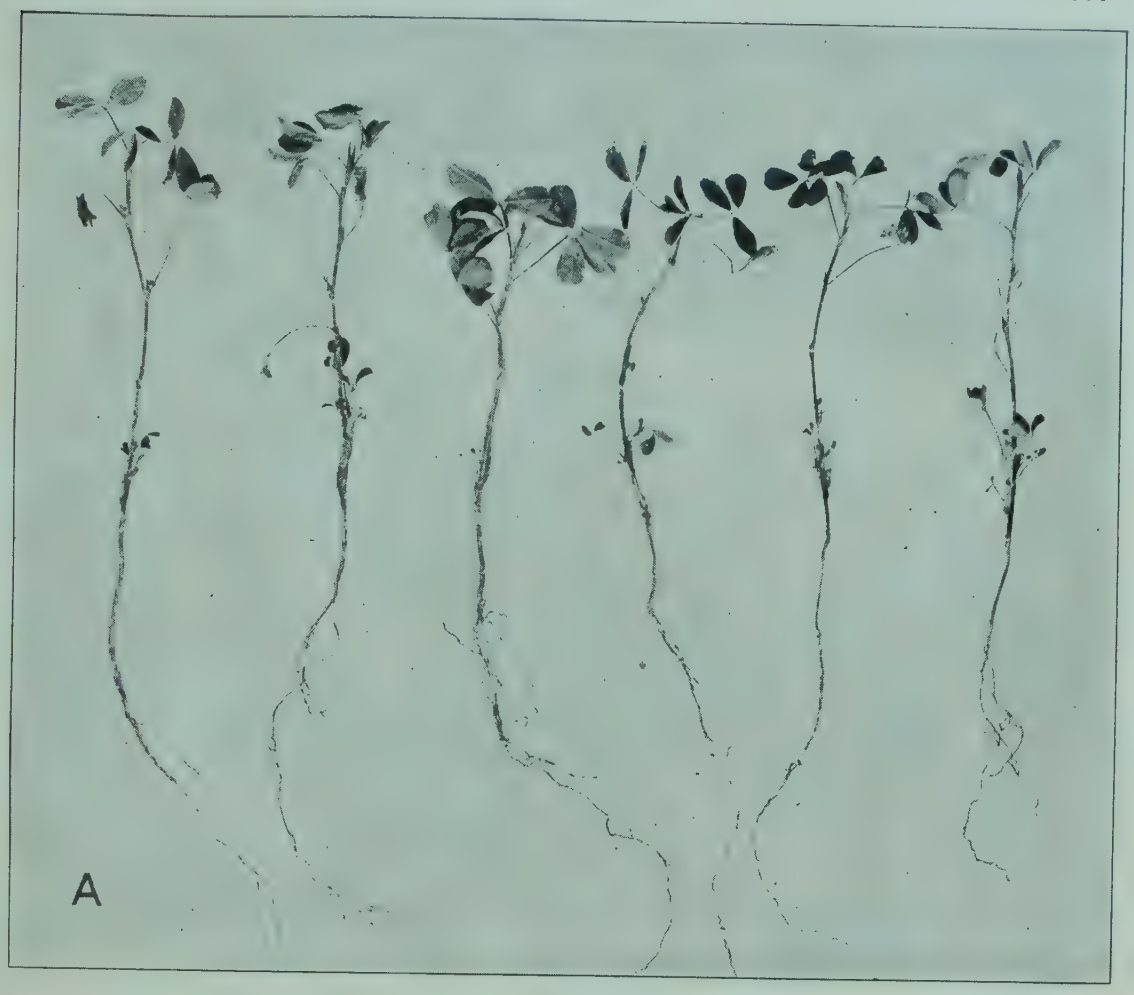
Three series of small flats were sown with seed of Peruvian, Kansas, Grimm, and Turkestan alfalfas, and *Medicago falcata* on January 20, 1921. One series was allowed to have the effect of the normal day, one was given the effect of a day shortened by the use of a light-excluding box placed over the flat at 4 p. m. and allowed to remain until 9 a. m., and one was given the effect of a day lengthened by turning on an incandescent electric light at dusk and turning it off at 11 p. m. The seedlings grown under the normal and the shortened day behaved essentially the same as those from sowings made in the early fall at Arlington. In height, erectness, and lack of branching the varieties ranged as follows: Peruvian, Kansas, Grimm, Turkestan, *M. falcata*. Seedlings under the lengthened day showed striking differences between the varieties but behaved in practically the reverse order from those under the normal and the shortened day. At the end of the first month the seedlings of *M. falcata* and Grimm exceeded those of the Peruvian and Kansas varieties in height. They were also quite as erect and unbranched.

From these experiments it is evident that it is the day-night relation of alfalfas and not their reaction to temperature that causes the development of marked differences between the seedlings of the various varieties when seed is sown at certain times of the year. By the proper control of the length of day, which can be accomplished in the greenhouse at any time of the year with little trouble or expense, and by the method used in these experiments it is easily possible to distinguish between seedlings of the commercial groups of alfalfas. The tests can be so quickly and easily made that the method is offered especially to Experiment Station workers and those engaged in alfalfa seed certification work as a means of assisting in the identification of the various lots of seed.

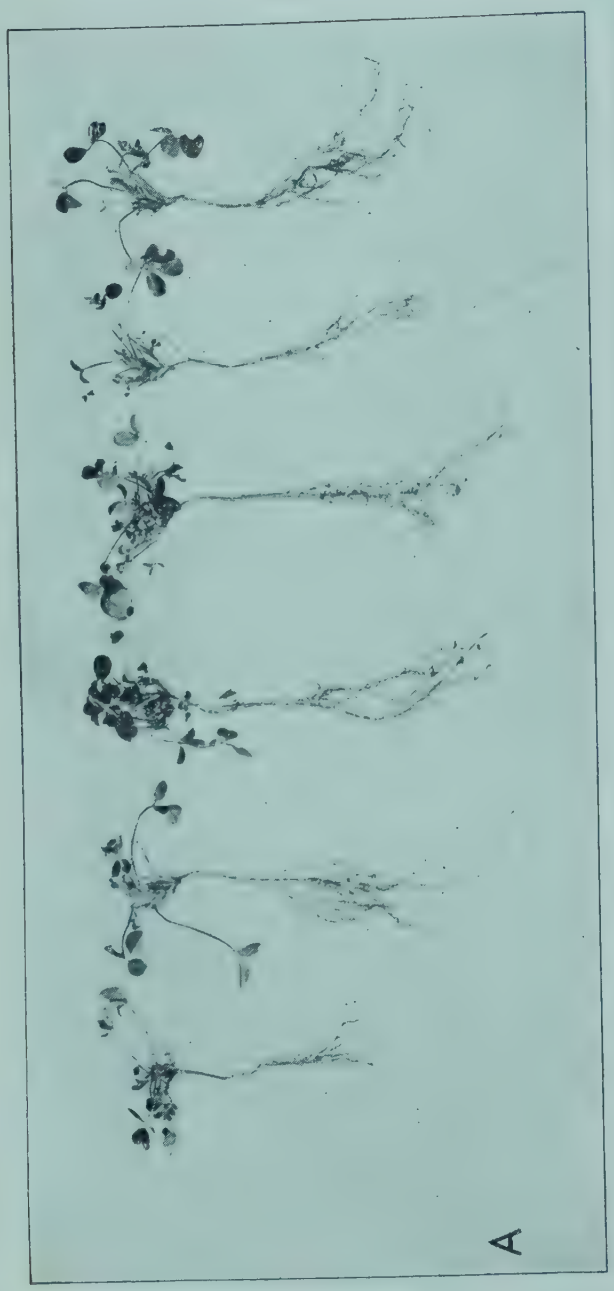
PLATE III

A.—Peruvian alfalfa sown at Arlington, Va., September 6. Photographed December 5, 1916.

B.—Kansas alfalfa sown at Arlington, Va., September 6. Photographed December 5, 1916.


A

B


A

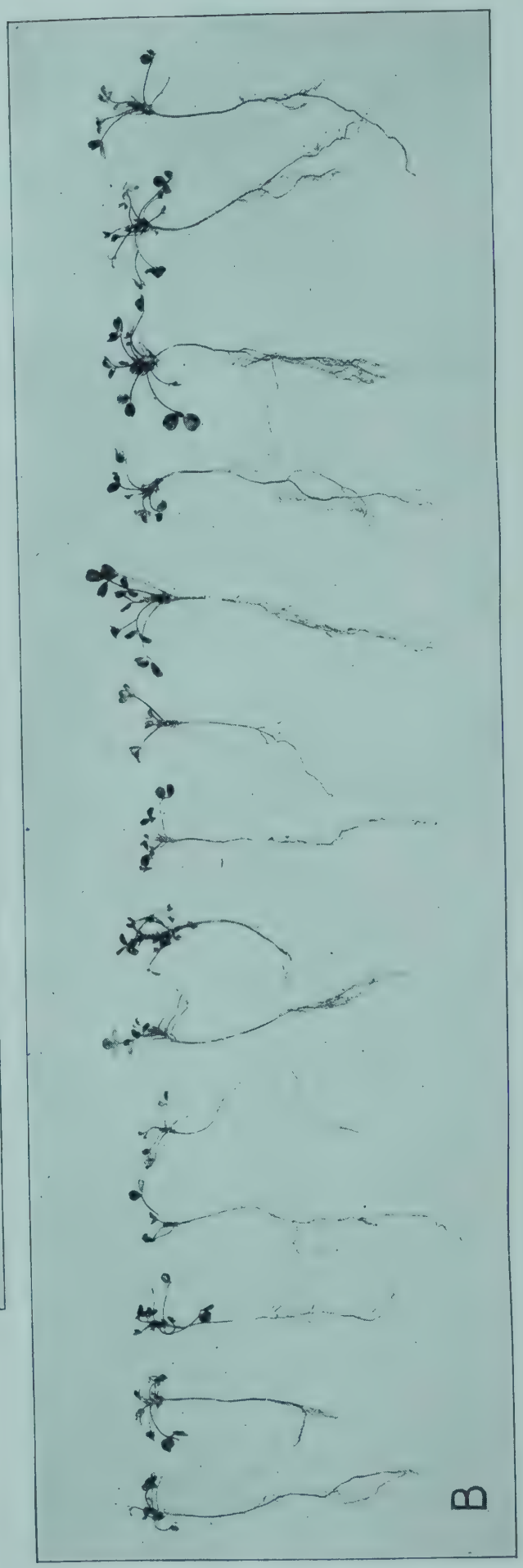

B

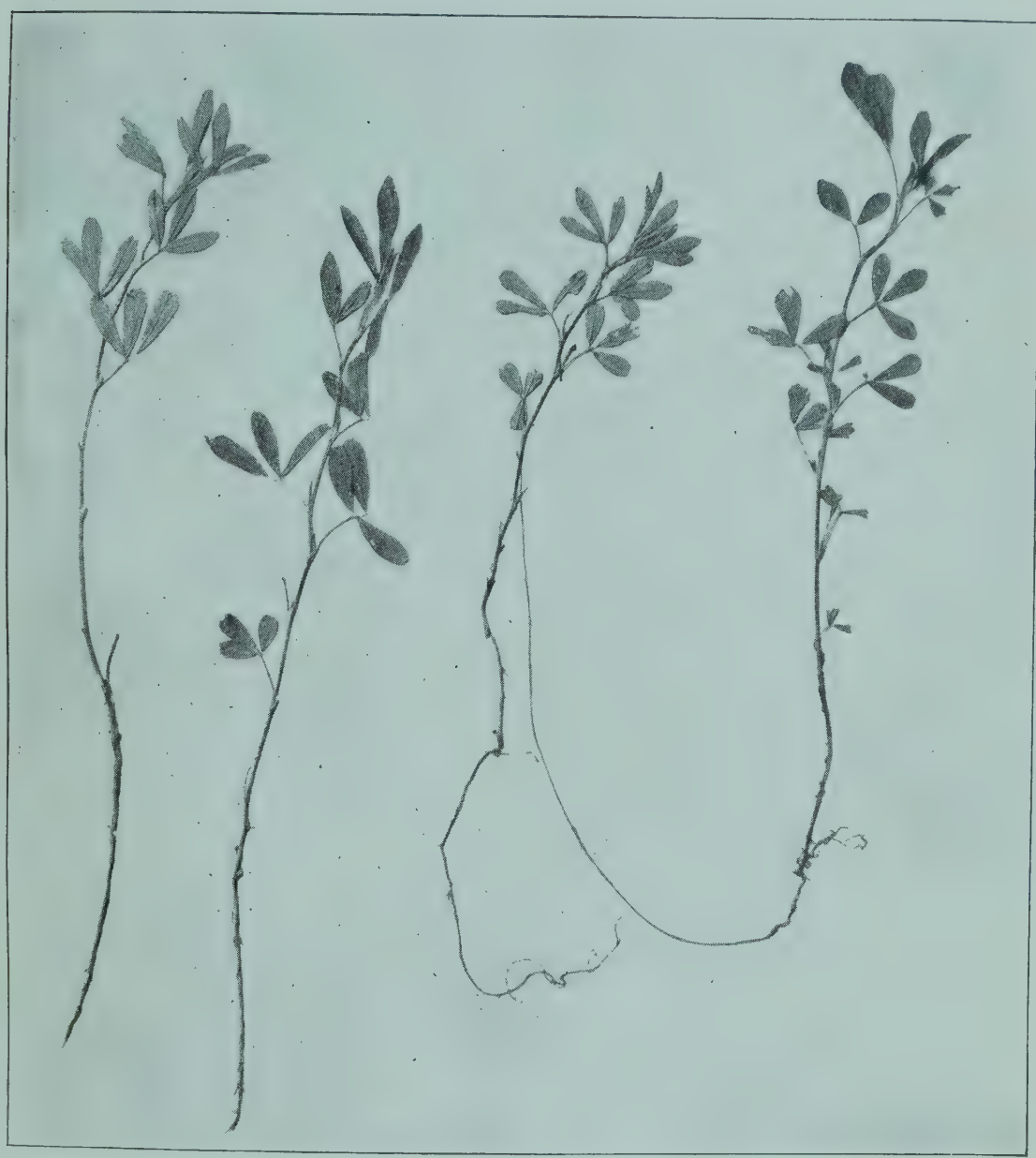
PLATE 112

A.—Grimm alfalfa sown at Arlington, Va., September 6. Photographed December 5, 1916.

B.—*Medicago falcata* sown at Arlington, Va., September 6. Photographed December 5, 1916.

PLATE 113

Peruvian alfalfa sown at North Ridgeville, Ohio, May 3. Specimens taken July 1, 1919.



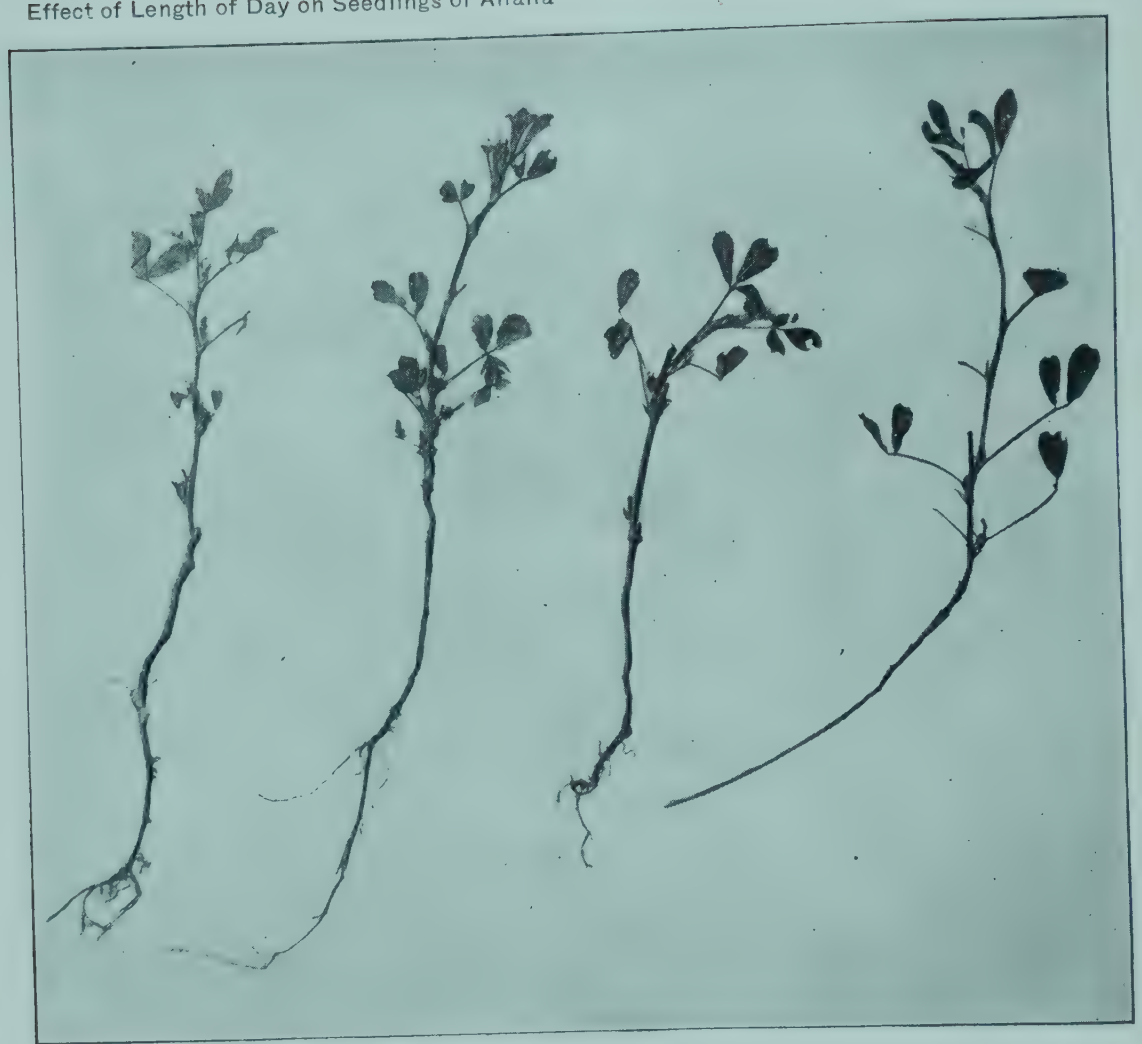


PLATE 114

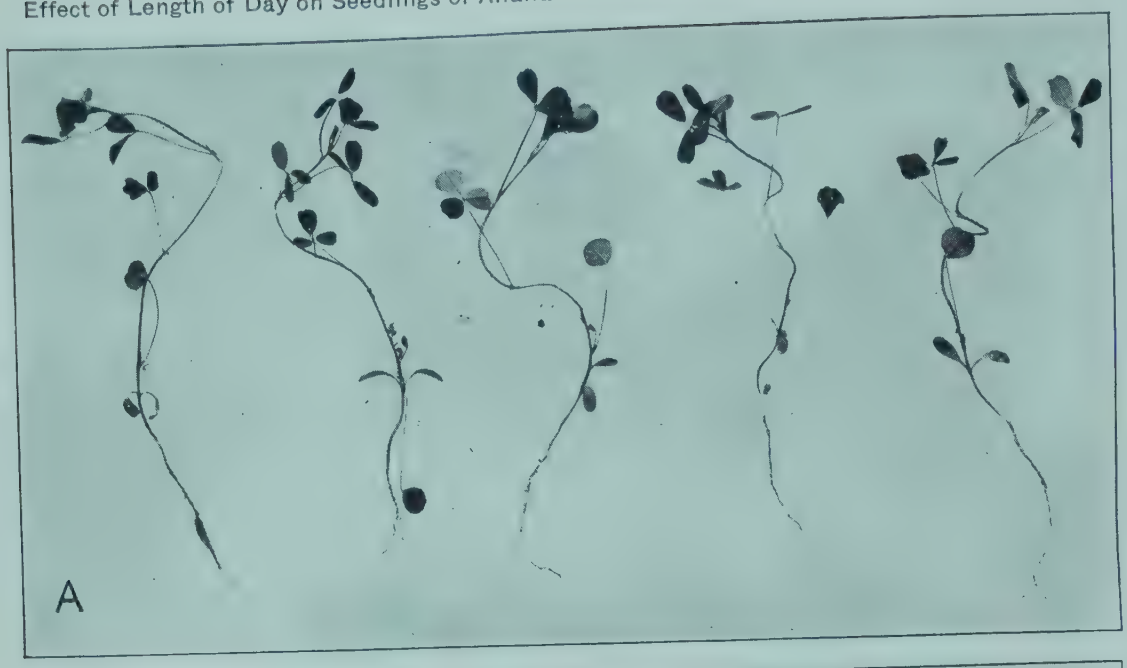
Kansas alfalfa sown at North Ridgeville, Ohio, May 3. Specimens taken July 1, 1919.

PLATE 115

Grimm alfalfa sown at North Ridgeville, Ohio, May 3. Specimens taken July 1, 1919.

Effect of Length of Day on Seedlings of Alfalfa

PLATE 115


A

B

C

PLATE 116

Peruvian alfalfa sown in greenhouse January 20. Photographed March 3, 1921.

A.—Grown under normal day with added illumination from a 100-watt electric light from dusk until 11 p. m.

B.—Grown under normal day.

C.—Exposed to daylight from 9 a. m. to 4 p. m.

PLATE 117

Kansas alfalfa sown in greenhouse January 20. Photographed March 3, 1921.

A.—Grown under normal day with added illumination from a 100-watt electric light from dusk until 11 p. m.

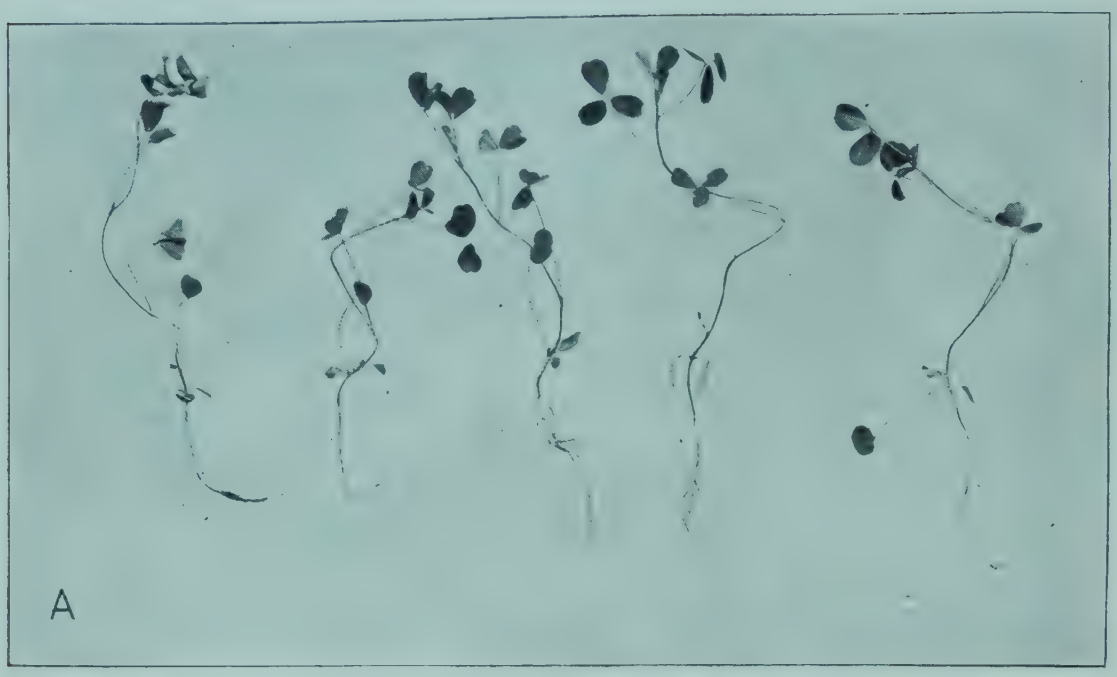
B.—Grown under normal day.

C.—Exposed to daylight from 9 a. m. to 4 p. m.

A

B

C


A

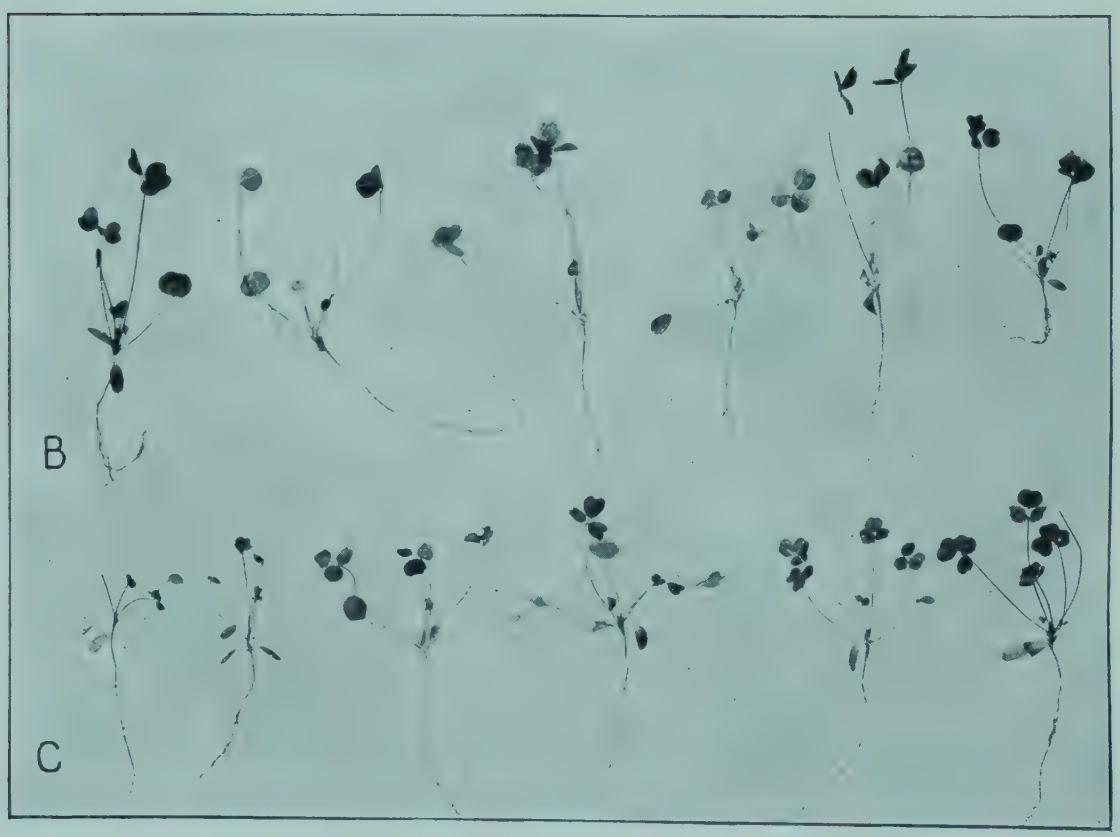

B
C

PLATE 118

Turkestan alfalfa sown in greenhouse January 20. Photographed March 3, 1921.

A.—Grown under normal day with added illumination from a 100-watt electric light from dusk until 11 p. m.

B.—Grown under normal day.

C.—Exposed to daylight from 9 a. m. to 4 p. m.

PLATE 119

Grimm alfalfa sown in greenhouse January 20. Photographed March 3, 1921.

A.—Grown under normal day with added illumination from a 100-watt electric light from dusk until 11 p. m.

B.—Grown under normal day.

C.—Exposed to daylight from 9 a. m. to 4 p. m.

A

B
C

A

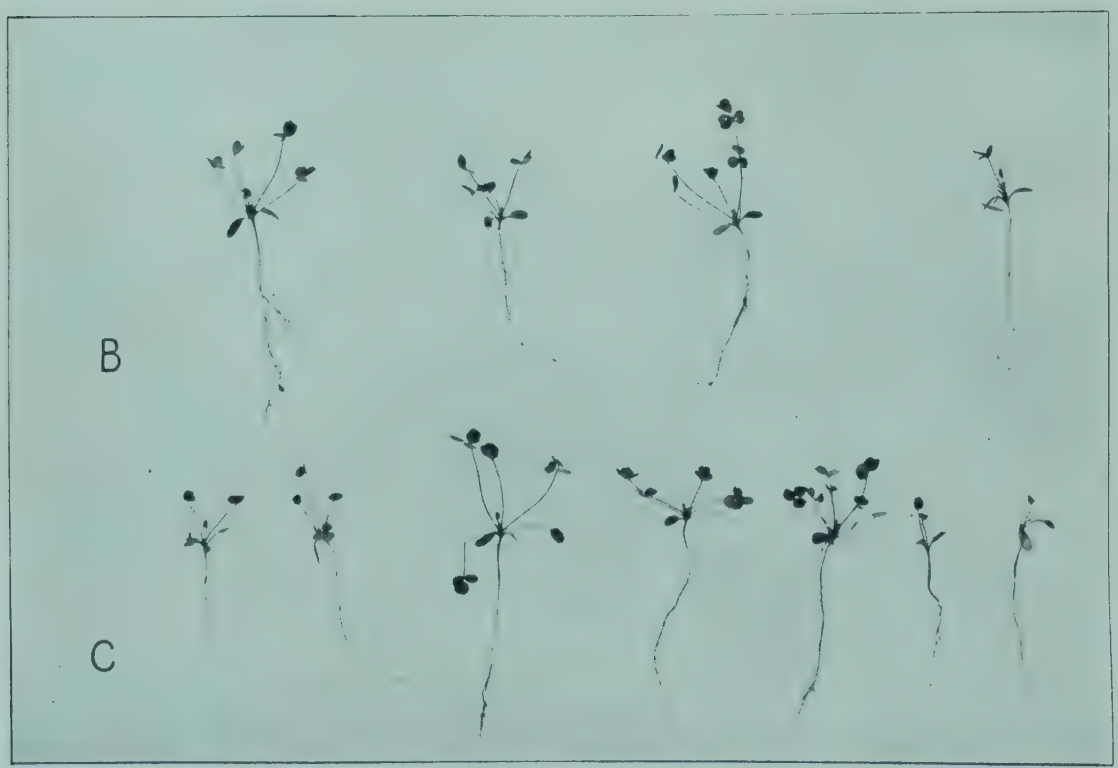

B
C

PLATE 120

Medicago falcata sown in greenhouse January 20. Photographed March 3, 1921.

A.—Grown under normal day with added illumination from a 100-watt electric light from dusk until 11 p. m.

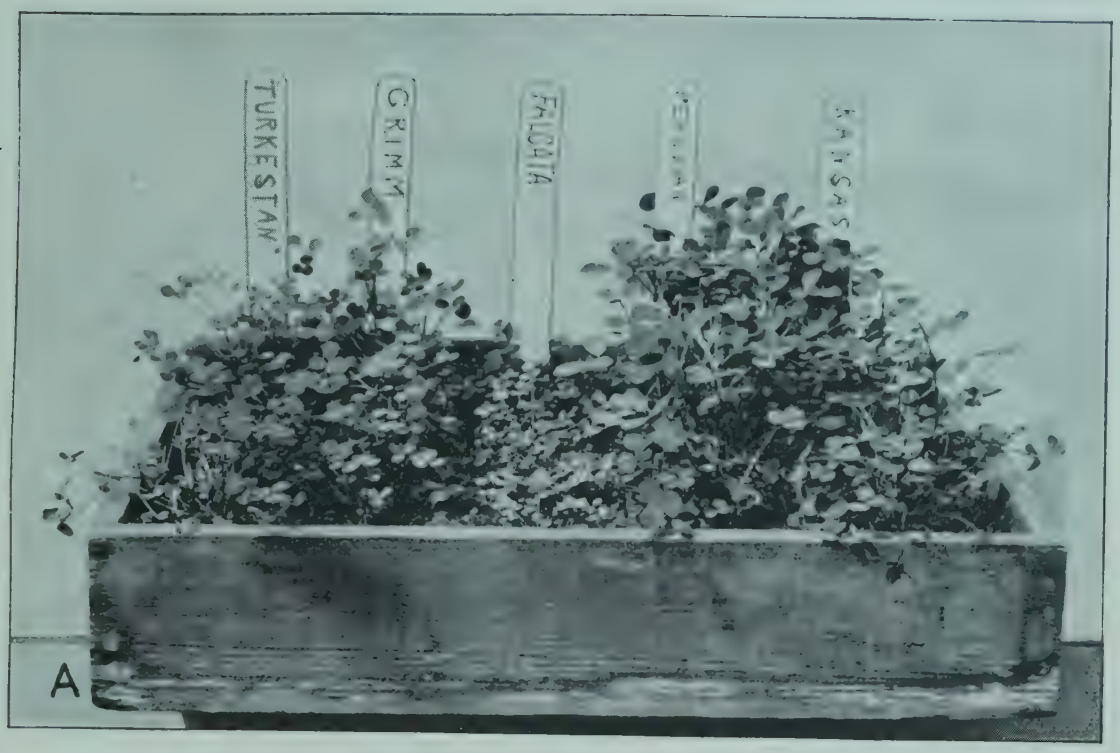
B.—Grown under normal day.

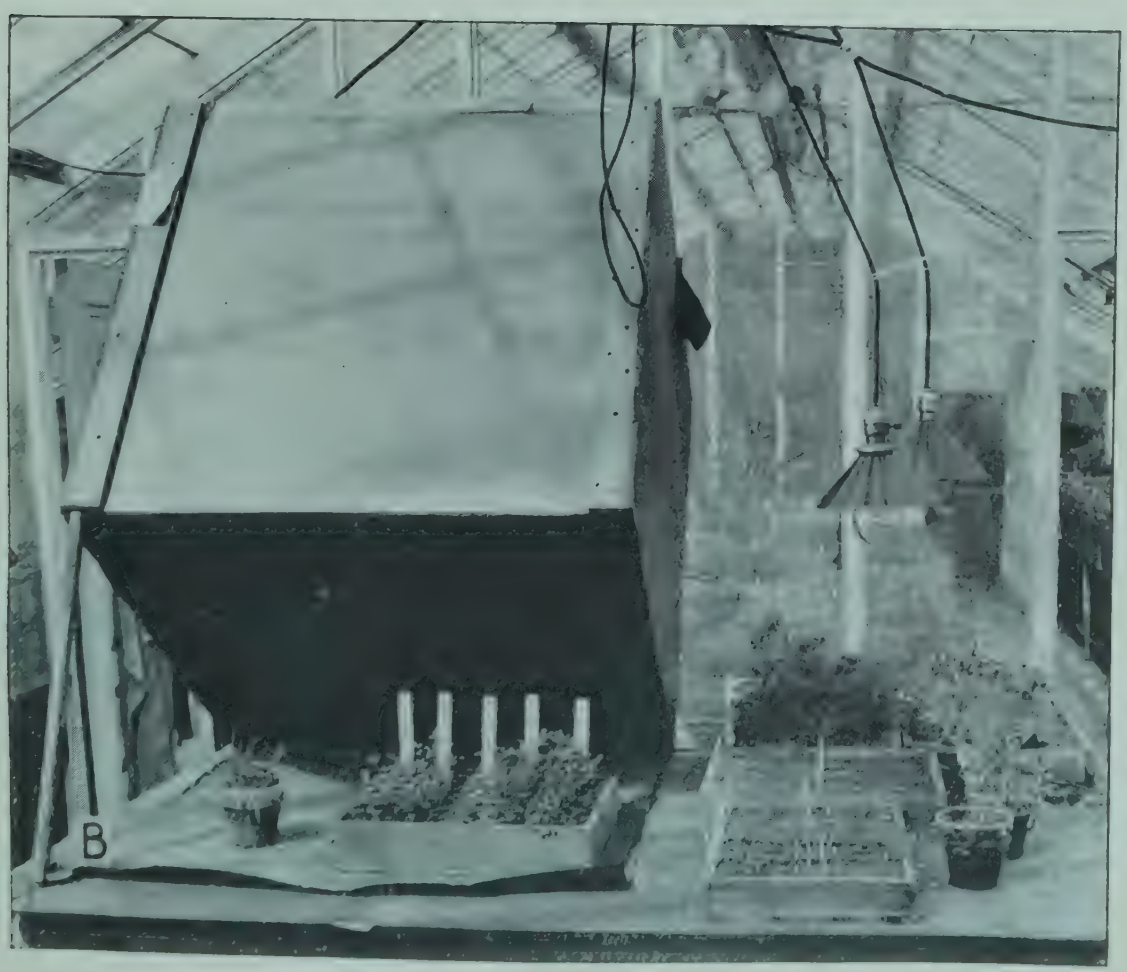
C.—Exposed to daylight from 9 a. m. to 4 p. m.

PLATE 121

A.—Alfalfa seedlings showing the effect of a short day on the growth of different varieties. Seed was sown in the greenhouse January 20, and seedlings were exposed to light from 9 a. m. to 4 p. m. Photographed March 22, 1921.

B.—Equipment used in determining the effect of different lengths of day on the seedling characters of alfalfa varieties.


TURKESTAN
GRIMM
FALCATA
KANSAS
A


B

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STUDIES IN THE PHYSIOLOGY OF PARASITISM WITH SPECIAL REFERENCE TO THE SECRETION OF PECTINASE BY RHIZOPUS TRITICI

By L. L. HARTER, *Pathologist*, and J. L. WEIMER, *Assistant Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

As a result of investigations of the relationship existing between a parasite and its host some knowledge has been obtained regarding the manner in which the infection takes place, as well as the way in which the host responds to the attack of the parasite. That some hosts are resistant to the attack of certain fungi or bacteria and not to others is well known, but why they are resistant is a subject on which there is altogether too little information. Furthermore the number of different hosts which may be parasitized by some of the so-called specialized parasites is at most only a few. The mode of infection is fairly well understood, but why a particular fungus is restricted to one or a few different hosts is not clear. On passing to parasites of a lower order the number of hosts which a particular fungus may attack is, in some cases at least, much larger. It is a well-known fact that some fungi can produce disintegration of the host tissues only after a wound has been made through which they can enter, while others have the power to penetrate the epidermis either mechanically or by the dissolution of the epidermal cell walls by enzymic action. There are, therefore, all degrees of resistance and susceptibility which apparently are dependent upon certain properties peculiar to either the host or the fungus. In other words, if a fungus can overcome the resistance of one host and not that of another it must be that the one possesses properties which the other does not. However, the experimental data at hand are altogether too limited to justify any sweeping generalizations as to what constitutes parasitism.

Rhizopus tritici Saito belongs to a large physiological group of fungi which are characterized by their ability to destroy the host quickly and to "act in advance" of their growth. It is assumed, and the evidence of the writers and of others seems to justify the assumption, that this "action in advance" is due to an enzym secreted by the fungus which

has the power to dissolve certain constituents of the cell walls so that coherence is lost.

The fungi belonging to this group kill rapidly and by destroying the middle lamellae transform the tissues into a mass of more or less isolated cells together with a large amount of a clear, somewhat colored liquid inclosed by the skin. The fungus secretes a substance which diffuses out of the hyphae into the tissue of the host and kills often several cells in advance of the fungous threads. The mycelium then must derive its nourishment from the dead material in which it is embedded. No doubt these fungi, since they live on the dead material thus produced, would be classed by some as saprophytes; nevertheless, they differ from true saprophytes in that they can attack and destroy living organisms. In this sense they are parasites.

The dissolution of the middle lamella has been demonstrated by several investigators to be due to a substance which is enzymic in nature. This enzym has been considered by most investigators to possess two rather distinct types of action on the cells of the host: first, a killing action on their protoplasmic contents, and, second, a dissolution of certain of the constituents of their cell walls. The writers at present will deal only with the latter.

The enzym which acts upon the pectic compounds of the cell walls, causing loss of coherence, has been designated by some investigators as pectinase and by others as pectosinase (*17*).[1] If it had been definitely proved that the middle lamella was largely composed of pectase the enzym which acted upon it should properly be designated as pectosinase. However, since the composition of the middle lamella has not been definitely determined and since the term pectinase is so widely used in the literature, the writers have employed it to designate the enzym secreted by *Rhizopus tritici* which dissolves the middle lamellae and produces what is termed maceration.

HISTORICAL

There is a considerable amount of data published which bear directly or indirectly on the physiology of parasitism, but only those which are germane to this phase of the subject will be considered here. DeBary (*1*) was probably among the first to detect the activity of a fungous extract in dissolving the cell wall. He showed that the drops which exuded from the sclerotia of *Sclerotinia libertiana* Fuckel., as well as the juice expressed from decayed plant tissues, contained a substance of the nature of a ferment which was thermolabile and possessed the power of dissolving certain constituents of the cell walls. He observed differences in the activity of the boiled and unboiled extracts, but he was not able to determine whether or not the entire action of the extract was due to one or

[1] Reference is made by number (italic) to "Literature cited," p. 624–625.

more substances. Similar results were obtained by Ward (*16*) and Kissling (*11*).

The work of Ward calls for special consideration. He grew Botrytis in culture and used the liquid on which the fungus grew, as well as a watery extract of the mycelium, to macerate living material and came to the conclusion that the entrance of the hyphae into the tissue depends upon the excretion of a ferment which dissolves the cellulose. Maceration was in most cases slow, which was probably due to the fact that cultures 3 weeks or more old were used. Further observations of Ward led him to conclude that the ferment sometimes accumulates to such an extent at the tips of the hyphae that the cellulose of the latter is dissolved and the ferment and protoplasmic contents of the cells pass out in the form of small droplets.

Nordhausen (*12*) used chiefly the extract from old mycelium, and came to the conclusion that the action on plant tissue was both enzymic and toxic in nature. He did not exclude the possibility that oxalic acid which was secreted by Botrytis might play a part. Behrens (*2*), on the other hand, used the expressed juice from fruits that had been decayed by certain fungi. In one case he employed the expressed juice of a pear infected with *Mucor stolonifer* Ehrb. and in another that of an apple infected with *Penicillium luteum* Zukal. He found these juices to be toxic to plant tissues even after boiling, and from these results he concluded that the toxic substance was neither volatile nor enzymic. In one case the fungus was allowed to grow three months before the extract was obtained.

Smith (*14*) compared the mycelial extract of Botrytis with weak solutions of oxalic acid on stems of lettuce. He found that the oxalic acid alone induced a macerating effect on the tissue similar to that of the extract of the fungus material, and on the basis of these results came to the conclusion that the dissolution of the cell walls noted is due to the oxalic acid secreted by the fungus.

So far as bacteria are concerned Potter (*13*) and Van Hall (*8*) found that *Pseudomonas destructans* Potter and *Bacillus omnivorus* V. Hall, respectively, produced enzyms which were able to dissolve the constituents of the cell walls of plant tissues so that coherence was lost. Potter worked both with the expressed juice of a turnip rotted with the organism and with the solution on which the bacterium had grown. He claims that a toxic substance was produced, since the boiled expressed juice from a rotted turnip caused the death of the cells of a raw turnip. He found that the organism when grown in Pasteur's solution produced oxalic acid which acts as a powerful toxic agent. It is claimed that the oxalic acid then acts as a toxin in killing the cells and that it may also play some part in the dissolution of the middle lamella. In this direction the most important work with bacteria is that of Jones (*10*), who carried on extensive researches with *Bacillus carotovorus* Jones. He found that this organism

secreted an enzym—pectinase—which has no action upon lignified or cuticularized walls, but which has the power to dissolve the middle lamella considerably in advance of the invasion of the bacteria.

Spauding (*15*) observed that in the last stages of decay produced by *Lenzites sepiaria* Fr. the middle lamellae have disappeared. Zeller (*19*) found that the same wood-destroying fungus produced a substance called pectinase capable of dissolving the middle lamella of carrots and potato disks, coherence of the tissue being entirely lost after 42 hours in an extract of the fungus powder.

Probably the most important contributions to the physiology of parasitism, at least as regards the intimate relations of the host to the parasite concerned, are those of Brown (*4*, *5*), Dey (*6*), and Blackman and Welsford (*3*).

It was shown that *Botrytis cinerea* Pers. acts in advance of its growth and that the active principle is of the nature of an enzym which dissolves certain constituents of the cell walls. It was further shown that the death of the protoplasmic contents of the cells was subsequent to the maceration of the plant tissues. To what the death of the cell is due appears to be a controverted point. Brown has been unable so far to identify two distinct principles in extracts of Botrytis mycelium. On the other hand, De Bary's (*1*), Nordhausen's (*12*), and Behrens' (*2*) conclusions seem to argue in favor of the presence of both an enzymic and toxic principle. Brown (*4*) showed that there was no dissolution of the underlying host tissues when extracts of the mycelium were placed on the unwounded surface of the host, and Blackman and Welsford (*3*) showed that the penetration through the unbroken surface of the host was accomplished by mechanical pressure. Similar results were obtained by Dey (*6*) with *Colletotrichum lindemuthianum* (Sasc. and Magn.) Bri. and Cav.

A résumé of the literature shows that various investigators have drawn quite different comclusions from the results of their experiments. Perhaps criticism of some of the earlier investigations might be justified on the grounds that they failed to describe their methods in sufficient detail to enable the reader to evaluate their results correctly. Furthermore, some of the conclusions have been drawn from the results obtained by the use of extracts of mycelium grown for a long time in culture, in some cases three weeks or more. It is probable that the material used had lost much of its power to dissolve the middle lamella, since Brown (*4*) showed that the macerating principle was most active in young and vigorously growing hyphae of *Botrytis cinerea* 1 to 2 days old. The writers also have found that the intracellular pectinase of Rhizopus is greatest in young mycelium. Harter (*9*), Dox (*7*), Young (*18*), and others showed that diastase, inulase, and other enzyms were more abundant in the hyphae just preceding the fruiting stage. It would, therefore, seem that young cultures should be employed if the maximum efficiency is to be obtained from the use of the hyphae.

METHODS OF EXPERIMENTATION

The investigations as outlined involved a study of the macerating action (*1*) of the enzym contained in the hyphae and (*2*) of that exuded from the mycelium into the culture solution. Brown (*4*) worked principally with the extract of the fungous material, although he domonstrated the excretion of the active principle into the culture medium. While offering no criticism upon the employment of the fungous extract, the writers have carried out macerating experiments with the solution on which the fungus grew as well as with the powdered hyphae. The use of the solution offered no mechanical obstacles, since to obtain fungus material the organism was grown on a liquid medium. Furthermore, in view of the fact that the macerating action is due to an enzym which diffuses out of the hyphae, it seemed to the writers that the macerating power of the solution should be studied concomitantly with that of the hyphae.

CULTURE MEDIUM

Since both the intracellular and extracellular pectinases were to be studied, a liquid medium which would support a rapid and luxuriant growth in a relatively short time and which would be available at all times of the year so that the investigations could be carried out without interruption was found to be preferable. The enzyms which diffuse out from the hyphae become dispersed throughout the medium, and after the liquor is freed of the mycelium it is ready for the macerating experiments. The medium which was finally employed was a decoction made from sweet potatoes prepared by the following formula: To the peeled potatoes add double the weight of water, steam for one hour, then squeeze out the liquid through gauze; steam a second time, filter by suction through absorbent cotton, add the required amount to flasks, plug with cotton, and autoclave for 20 minutes at 15 pounds pressure. The resulting solution is practically free of cellular structures but does contain some starch and some sugars.

In this medium a felt or mat composed of fungous threads, floating for the most part on the surface, which can readily be lifted from the liquid, is formed in from one to two days.

In view of the fact that a large quantity of mycelium was frequently necessary, 2-liter Erlenmeyer flasks containing 250 cc. of the decoction were used. If more than one flask was used the hyphae from all of them were made into one compound sample. The maceration of the raw sweet-potato disks was carried out with a weighed amount of this sample suspended in a definite amount of distilled water. Accordingly the solutions from all the flasks on which the fungus grew were collected into one flask, thoroughly mixed, and the maceration was carried out with a measured quantity.

The flasks were inoculated with spores and bits of hyphae from stock cultures which were kept in a vigorous state of growth. Incubation was carried out in the dark at a constant temperature, the temperature used depending on the character of the experiment.

UTILIZATION OF THE MYCELIUM AND SOLUTION

At the end of the incubation period the fungus growth was removed, washed in running water for 15 minutes, the water squeezed out by hand, and then treated with acetone and ether, according to Dox's (7) modification of Albert and Buchner's method for preparing "Aceton-dauerhefe." After washing, the operation is essentially as follows: The mycelium is immersed for 10 minutes in a large excess of acetone and constantly stirred, the hyphae being torn apart by forceps at the same time. The material is then squeezed to dryness and immersed in a fresh supply of acetone for 2 minutes with constant stirring. It is again squeezed to dryness and stirred for 3 minutes in ether, after which it is laid out to dry. After the odor of the ether can no longer be detected, the mycelial mass is put in flasks and stored at 9° C. until required for use.

Except in so far as it was necessary to meet the requirements of special experiments maceration of raw sweet-potato disks was carried out by the use of 0.25 gm. of the mycelium ground to a powder in fine quartz sand. The sand used for grinding was thoroughly washed in distilled water and then burned in a crucible for two hours. The enzym powder was then transferred to 120-cc. Erlenmeyer flasks, and 25 cc. of distilled water were added. Several, usually five, raw sweet-potato disks 1 mm. thick and 15 mm. in diameter were placed in each flask containing the suspension of hyphae and sand. The disks were always cut from that part lying within the fibrovascular ring of the potato, since the tissue there is more uniform, being made up largely of thin-walled parenchyma cells with only here and there a vascular bundle.

The tissue inside the fibrovascular ring of different sweet potatoes has been observed to differ somewhat in character and to react differently to the macerating agent. In view of this fact each experiment was always conducted with disks taken from the same root. The potatoes were carefully selected for soundness of the internal tissue, and any showing cavities or pithiness were rejected. In a few preliminary experiments the air was removed from the disks by suction before they were put in the solution. However, this practice was found to be unnecessary and was finally abandoned after the results showed that the rate of maceration was neither increased nor decreased by the air in the tissue.

The flasks after the disks had been added were incubated at a constant temperature, and notes were taken of the progress of maceration from time to time. Loss of coherence of the tissue was regarded as complete when the disks offered no resistance to pull. In a determination of the

end point the personal factor naturally enters, but with experience it may be determined within relatively narrow limits.

Immediately upon the removal of the fungous felt the solution was filtered through absorbent cotton to remove the remaining threads of hyphae. Cotton was used in preference to filter paper for reasons to be pointed out later. Except for special experiments to be discussed later the cultures were grown for only three days so that usually no, or only very few, spores were produced. Raw sweet-potato disks of the type mentioned above were immediately suspended in the filtered solution, incubated at the desired temperature, and records made of the progress of maceration. By this method of manipulation it is not unlikely that some spores, if produced, and some foreign organisms found their way into the solution. Pure cultures of the organisms were grown under sterile conditions, but as soon as the fungous felt was removed it as well as the solution doubtless became contaminated by foreign organisms from the air. The extracts and the solutions were so powerful in pectinase that maceration of the tissues was complete before these organisms could influence the results appreciably. In this connection it may be stated that in no case have contaminating bacteria been found to exercise any macerating action on raw disks of sweet potatoes. In cases where the macerating action was prolonged beyond two or three hours the surface of the liquid was covered with toluol. In a number of trial experiments it was found that no foreign organisms could be isolated from solutions which had been well shaken with toluol.

Other investigators in this field have carried out most of their experiments by the use of an extract of the fungous material. This is usually obtained by grinding the mass of hyphae, suspending it in water for a definite length of time, then filtering out the fungous débris. This method was not followed by the writers for reasons which will be made clear later. The ground mycelium was suspended in the water and formed a part of the system to which the disks were added. Several objections may be offered against the extraction of the hyphae. First, it allows foreign organisms, which doubtless are introduced during the process of manipulation, to multiply. Second, a considerably longer time is required to complete an experiment and, third, all the enzym may not be extracted and some of it may be removed by the filter paper.

EXPERIMENTAL DATA

PECTINASE FORMATION BY RHIZOPUS TRITICI

At the outset of the storage-rot experiments it was sometimes difficult to obtain a culture of Rhizopus from rotted sweet potato, although there was an abundance of evidence that this fungus caused the decay. In order to avoid foreign organisms the plate plantings were made from partially decayed tissue adjacent to the healthy zone. Plates so made

were frequently sterile, which at once suggested the possibility that there was disintegration of the tissue or "action in advance" of the growth of the hyphae. Cultures made a little more remote from the healthy tissue usually gave a pure culture of Rhizopus. Following up the clue suggested by the plate plantings, preliminary macerating experiments were made with the solution on which the fungus grew and with extracts of the hyphae, both of which resulted in the complete disintegration of raw sweet-potato disks within a few hours.

Literally hundreds of macerating experiments have been carried out with the enzym contained in the hyphae and with that exuded into the substratum. Because of the large number of experiments detailed elsewhere dealing with special phases of the subject and which contribute additional evidence of the occurrence of pectinase in the mycelium and in the solution on which it grew, only a résumé of the process of maceration will be described here. The raw sweet-potato disks, usually five, of uniform size (1 mm. thick by 1.5 cm. in diameter), were dropped into the solution or into the hyphal suspension, as the case might be, both of which were prepared according to methods already described. The incubation was usually carried out at 37.5° C., although higher or lower temperatures, depending upon the object of the experiment, were sometimes used. In some of the more powerful preparations a change in the appearance of the disks frequently took place in from 10 to 15 minutes after they were added to the solution. The disks became somewhat cupped or twisted in shape and slightly crisp, brownish in color, probably due to oxidation. Approximately ½ hour thereafter, depending somewhat upon the macerating power of the enzym, the disks became flaccid. This was followed by softening of the tissue at the surfaces and edge. The progress of the softening continued gradually until the disks were softened throughout so that they offered no resistance to pull. A microscopic examination of completely macerated tissue shows that the cells are separated along the line of the middle lamellae but that the cells themselves remain intact.

The time required to macerate the disks completely varied from about 1 to 5 hours. Several factors were found to influence the rate of maceration. No two preparations could be regarded as exactly alike, although the cultures were grown and the solutions and hyphae treated thereafter under as nearly identical conditions as possible. Again it was found that the end point of maceration varied with the different disks in the same system, although they were all cut from the same sweet potato and from the same portion of the potato. It was found, therefore, that something must be left to the judgment of the observer. However, after considerable practice in the determination of the end point it is possible to determine it within relatively narrow limits. These controlled and carefully conducted experiments show that *Rhizopus tritici* produces a powerful intracellular and extracellular pectinase which can dissolve

the middle lamellae of sweet-potato disks so that the cells fall apart without the cells themselves undergoing any apparent mechanical alteration.

INFLUENCE OF THE AGE OF THE MYCELIUM ON THE RATE OF MACERATION

Brown (*4, I*) showed that the young, growing hyphae of *Botrytis cinerea* contained a more powerful enzym than old hyphae, and he used for most of his work an extract of the fungus grown for only 24 hours. Other investigators have obtained similar results for other enzyms of various fungi. It appears to be generally agreed that the most powerful extract is obtainable from young or comparatively young hyphae, but it can not be expected that all fungi will show the same limitations as regards the length of time for growth. This being a question of considerable importance with respect to much of the future investigations in this direction, experiments were planned and carried out in considerable detail for the purpose of determining at what period in the growth of the organism the most powerful extract could be obtained from the hyphae, and likewise when the maximum amount of the enzym was present in the solution. While it may be argued that comparative studies do not necessarily require that the work be done with hyphae and solutions containing the maximum amount of enzym, there are several reasons why it is desirable to do so. First, the time required to complete maceration is shortened so that the experiments may be completed within a single day. Second, a short period of maceration eliminates to a large extent the influence that might be exercised by the introduction of contaminating organisms. Third, infection is essentially dependent upon the extracellular enzym of young hyphae, at least in so far as enzyms play a part.

To determine the period of maximum content of enzym in the hyphae and in the solutions, 50 flasks containing sweet-potato decoction and 36 inoculated with spores of *Rhizopus tritici* were prepared. All the flasks were incubated at 37.5° C. in the dark. At the end of each 24-hour period, with exceptions to be noted in the following table, 4 of the flasks (3 inoculated and 1 not inoculated) were taken from the incubator, the fungous felt was removed, and the solution was filtered through absorbent cotton. The hyphae from the inoculated flasks were treated in the usual way with acetone and ether, and after drying the fungous material was made into one compound sample. Duplicate 0.25-gm. samples of the hyphae were weighed out, ground in sand, suspended in 25 cc. of distilled water, and used for the maceration of the sweet-potato disks. A water blank containing no enzym powder was used as a control. The incubation temperature was 37.5°. Records were made from time to time of the progress of maceration.

The contents of one of the three flasks on which the fungus had grown were steamed to deactivate the enzym, and together with one flask not

inoculated they served as a control. Maceration was carried out at 37.5 C. The results of these experiments are shown by Table I.

TABLE I.—*Time in hours required by the mycelium and the solution on which it grew to macerate sweet-potato disks after growth in sweet-potato decoction for stated intervals of time*

	Hours required to macerate after growing in culture—											
	1 day.	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.	10 days.	11 days.	12 days.	13 days.	16 days.
Powdered hyphae:												
Enzym powder.....	3.0	4.00+	4.5	4.5	4.75	5.5 +	5.5 +	5.5+	5.25	7.0 +	8.5	19.0
Water (control), no maceration.												
Solution:												
Not steamed.....	3.0	1.75	1.75	1.75	1.75	1.75	1.75	2.0+	1.5	2.25+	4.5	6.0+
Steamed (control), no maceration.												
No fungous growth (control), no maceration.												

From Table I it is seen that the macerating power of the hyphae per unit of dry weight is greatest on the first and second days of growth and becomes gradually weaker thereafter. The enzym of the solution, on the other hand, is equally powerful from the second to the seventh day of growth, inclusive. The writers have arbitrarily adopted three days as the standard length of time for growth. Somewhat discordant results were obtained in the steamed solutions and in the solutions on which the fungus was not grown. No maceration was apparent in such solutions at the end of the time required to macerate completely the disks in the solutions containing the active enzym. On the other hand, in some cases if they were exposed for some hours longer maceration was evident, and it was often completed in 24 hours.

RELATION OF VARIOUS FACTORS TO MACERATION

The writers have previously discussed the method used in carrying out macerating experiments with the enzym contained in the mycelium and with that exuded into the substratum. Experimental data have also been submitted which show that *Rhizopus tritici* produces both a powerful intracellular and extracellular pectinase which dissolves the middle lamellae of raw sweet-potato disks in from two to five or more hours (Table I), depending upon the length of time the organisms grew in the culture. It was shown that the most vigorous enzym was contained in cultures 1 to 3 days old.

In investigations of this kind the methods used in the treatment of the fungous material or of the solution might conceivably greatly influence the activity of the enzym. To obtain reliable data it was, therefore, necessary to make a careful study of the physical, mechanical, and other factors involved in the various steps in the process of manipula-

tion to determine whether they altered the macerating power of the enzym, and if so, to what degree.

WASHING THE MYCELIUM.—The first step in the treatment of the fungous growth as finally adopted consisted in washing the felt in running tap water for about 15 minutes, squeezing out the water, and subsequently treating it with acetone and ether. Washing in water for this length of time did not remove any of the macerating principle from the mycelium, as is shown by the following experiments. Several fungous mats (two days' growth) about 6 inches in diameter were removed from the culture flasks and rinsed for 2 or 3 seconds in running water to remove some of the adhering culture fluid. The felts were then cut into two parts, one of which was washed for 15 minutes in flowing water. Both lots were subsequently treated with acetone and ether in the usual way. A comparison of the macerating power of the two lot of material was made by using 0.25 gm. of the fungous hyphae ground in sand and suspended in 25 cc. of distilled water. Several sweet-potato disks (1 by 15 mm.) added to the systems and incubated at the same temperature were macerated in an equal length of time.

ACETONE-ETHER TREATMENT.—The treatment of the mycelium with acetone and ether did not influence the macerating power of the hyphae, as was shown by a comparison of the corresponding halves of the same felts, one of which was dried over calcium chlorid in partial vacuum for five hours and the other treated in the usual way with acetone and ether. After the felts of each set were thoroughly dried they were mixed into a compound sample, and 0.5 gm. of the powdered mycelium of each was suspended in 25 cc. of distilled water. Sweet-potato disks (1 by 15 mm.) were macerated in equal time in an enzym powder of the same concentration for the two samples. From these results it would seem that the treatment of the mycelium with acetone and ether in no way influences the macerating power of the hyphae.

Probably the chief virtue in the acetone-ether method lies in the ease with which the dried mycelium can be handled subsequently. Rhizopus forms a very compact, leathery mat which is difficult to handle if allowed to dry before the threads are torn apart. The practice has been to pull the hyphal threads apart while the material is submerged in the acetone and ether, so that by the time the treatment is finished the threads are well separated and broken into short lengths. When dried the material is loose and cottony in general appearance, so that a quick and accurate weighing is possible.

GRINDING THE HYPHAE BY SAND.—A comparison of the rate of maceration by mycelium ground in sand with mycelium not so ground showed that, everything else being identical, the disks in the suspension of ground hyphae reached complete maceration a little ahead of those in the solutions with unground hyphae.

The quantity of sand used for grinding the mycelium does not influence the rate of maceration, as is shown by the results of comparative experiments in which several uniform lots of mycelium were ground with 0.25, 0.5, 1.0, 5.0, and 10.0 gm. of sand, respectively, and transferred to small Erlenmeyer flasks. To each flask were added 25 cc. of distilled water and immediately the usual sized sweet-potato disks. Maceration carried out at 37.5° C. was complete in all samples ground in sand in 3.5 hours. In the unground samples which served as controls maceration was complete in 4 hours.

TOLUOL AS AN ANTISEPTIC.—In experiments where a considerable length of time was required to complete maceration, about 2.5 cc. of toluol, or enough to cover the surface of the liquid, was used as an antiseptic. Usually antiseptics were not employed, since maceration was completed before foreign organisms became sufficiently abundant to influence the results materially. Sweet-potato disks immersed in pure toluol for 19 hours showed no evidence of maceration. The use of toluol as an antiseptic in flasks containing enzym powder did not retard the maceration of sweet-potato disks when compared with flasks to which no toluol was added, maceration being completed in each case in an equal length of time.

EXTRACTION OF THE HYPHAE.—The usual method in investigations of this kind is to extract the powdered mycelium with water for a given time, filter off the solid matter, and subject the disks or material to be macerated to the extract. Two objections may be offered to this method. First, it requires a considerably longer time to carry out the experiments and, second, all of the enzym may not be removed from the hyphae. A considerable number of experiments which need not be related in all their details were conducted to determine whether extraction of the hyphae was necessary or desirable. A comparative study was made of the rate of maceration of 0.25 gm. of hyphae extracted for 18 hours in 25 cc. of water at 9° and at 37.5° C., respectively, and of hyphae not previously extracted. The results of these experiments showed that the disks added to the unfiltered solutions and incubated at 37.5° in both the extracted and nonextracted mycelium were macerated in the same length of time.

FILTERING.—Since extraction of the mycelium was found unnecessary, filtering the solutions free of the mycelial particles and sand was not practiced. Suffice it to say in this connection that the several experiments conducted showed that filtering the solution through one and two thicknesses of No. 2 Whatman chemically prepared filter paper considerably weakened the extract. To just what the weakening of the enzym was due was not determined, but it is probable that some of the enzym was retained in and adhering to the fungous particles and sand which were caught by the filter paper.

On the other hand, filtering the solution on which the fungus grew gave different results. Although most of the fungous material could be lifted from the flask, filtering was necessary before one could be reasonably sure that no hyphae remained in the solution. Aside from the hyphae which were left in the solution, the liquid was reasonably clear and free of solid particles. To determine whether filtering removed any of the macerating principle, a number of experiments were made with solutions on which the organism had grown for about 40 hours at 37.5° C. The contents of two large flasks from which the fungous growth had been removed were mixed into one compound sample, one portion of which was filtered through a No. 2 Whatman chemically prepared filter paper, and another portion through a thick layer of absorbent cotton. The remainder served as a control, a portion of which was steamed 15 minutes to inactivate the enzym. Raw sweet-potato disks were then added to the different solutions and incubated at 37.5°. The results showed that the disks were macerated as quickly in the solution filtered through filter paper and cotton as in the unfiltered solution.

CENTRIFUGING.—Some investigators have removed the sand and fungus débris from the solution by centrifuging, a method well adapted to this type of work if an extract is required. This method is possibly open to the same objection as filtering the extract through filter paper in which it was shown that some of the macerating principle was removed. A comparison was made of the macerating power of the supernatant liquid of a centrifuged solution with that of the solutions of the same origin filtered through filter paper, through cotton, and not filtered. The experiment was carried out by the use of 0.5-gm. samples of hyphae ground in sand and extracted for 18 hours in 25 cc. of distilled water. At the end of 18 hours the contents of some of the flasks were centrifuged for 5 minutes, and the supernatant liquid was used for macerating experiments. The contents of some of the other flasks were filtered either through filter paper or through cotton. The remaining flasks were not filtered and served as controls. The results showed that maceration was complete in the solution not filtered or centrifuged in 3.5 hours, in the centrifuged solution in 4 hours, and in the filtered solutions in 5 hours.

RELATION TO SUNLIGHT.—Light is said to be injurious to certain enzyms. In view of the fact that most of the mechanical operations of the present investigations must be carried out in diffused light, in some cases the enzym powder being exposed to the light for a considerable length of time, it was thought advisable to determine what effect, if any, such exposure had on the macerating power of the enzym. These experiments were carried out by exposing for 2 hours lots of 0.25 gm. of hyphae to direct sunlight and diffused light and placing some in an incubator in which light was practically excluded. In some experiments

the mycelium was ground before it was exposed to the light. At the end of the period of exposure the powdered hyphae were placed in an incubator for 18 hours at 9° C. The mycelial powder was then suspended in 25 cc. of distilled water, and after sweet-potato disks of the usual type had been added, incubation was carried out at 37.5°. Records taken from time to time of the progress of maceration showed that the different light intensities used had no influence upon the macerating principle. Grinding before exposure to light was likewise without effect, maceration being completed in 4.5 hours in all cases.

QUANTITY OF SOLUTION.—It is desirable, other things being equal, to employ technic in investigations of this kind which will require the least possible time in manipulation. If the volume of the solution whose macerating property is to be studied should influence the rate of maceration, a measured quantity would be required for all comparative work. This would require time in measuring. Without going into the details of the results of this phase of the work it may be stated that there was no difference in the time required to macerate sweet-potato disks in a solution on which the fungus grew in volumes varying from 25 to 100 cc.

CONCENTRATION OF SOLUTION.—As would be expected, diametrically opposite results were obtained as regards the concentration of the solution, the rate of maceration being most rapid in the solutions containing the largest quantity of enzym powder.

OPTIMUM TEMPERATURE FOR MACERATION.—It is a well-known fact that temperatures below the optimum progressively retard the action of enzyms. Above the optimum, action is retarded more rapidly. With respect to the macerating principle found in *Botrytis cinerea*, Brown (5) showed that at 65° C. deactivation was practically instantaneous.

The investigations of the writers with respect to this subject were made with a solution obtained from six 2-liter flasks on which the fungus had grown for 48 hours at 37.5° C. The solutions were filtered through two thicknesses of cheesecloth and combined into one large receptacle and thoroughly mixed. Twenty-five cubic centimeters of this solution were pipetted into small flasks and exposed for 1 hour at 9°, 20°, 30°, 40°, 45°, 50°, 55°, and 60°, respectively, to bring to constant temperature. Raw sweet-potato disks were then added to the flasks, and notes were taken from time to time of the degree of maceration. At 9° maceration was completed in 3.5 hours, and the time was progressively shortened with an increase of the temperature up to the macerating optimum. At 60° the enzym was completely deactivated and no maceration took place. The optimum temperature has been difficult to establish, but it lies somewhere between 45° and 55°, and probably nearer 45° than 55°. If more refined methods were at hand for determining the end point the optimum might probably be more nearly established. Above 55°

deactivation of the enzym was very rapid, and below 45° its action was retarded. Experiments with the ground hyphae in which 0.25-gm. lots were suspended in 25 cc. of distilled water and exposed to the same temperatures as the solutions discussed above gave similar results. In recapitulation it may be said that the intracellular and extracellular pectinase bear the same relation with respect to the optimum temperatures for maceration.

SUMMARY

(1) *Rhizopus tritici* produces a powerful intracellular and extracellular pectinase when grown in sweet-potato decoction.

(2) The enzym is able to effect the complete maceration of raw sweet-potato disks so that coherence of the cells is entirely lost.

(3) The optimum temperature for maceration is between 45° and 55° C. At 60° deactivation of the enzym is nearly instantaneous; below 45° the activity of the enzym decreases simultaneously with the decrease in temperature.

(4) The maximum enzym content of the hyphae and the solution is attained in about 24- and 48-hour-old cultures, respectively.

(5) The volume of the enzym solution of a given strength does not influence the rate of maceration; the concentration of the enzym in the solution does.

(6) Exposure of the hyphae for 2 hours to direct sunlight does not affect its macerating power.

(7) Centrifuging to remove the sand and fungous débris slightly deactivates the enzym.

(8) Filtering the solution in which the powdered hyphae and sand are suspended through filter paper weakens the enzym; filtering the solution after the removal of the fungous felt does not reduce its strength.

(9) Extraction of the powdered hyphae for 18 hours in water does not increase the rate of maceration when compared with hyphae not extracted.

(10) Toluol may safely be employed as an antiseptic without impairing the action of the enzym.

(11) The quantity of sand used for grinding the hyphae does not influence the action of the enzym.

(12) The treatment of the hyphae with acetone for 12 minutes and ether for 3 minutes has no influence on the macerating action of the hyphae.

(13) Washing the hyphae in running water for 15 minutes has no influence on the action of the enzym.

(14) The results of these investigations indicate that work of this type involving a study of the relationship existing between a host and its parasite may throw some light on the important question of parasitism.

Rhizopus tritici belongs to a large group of organisms, incapable of themselves of penetrating the unbroken cells of the epidermis mechanically or of dissolving them with its enzyms. However, after it has once reached the tissues beneath the epidermis, it progresses with great rapidity. It, like certain other organisms, is characterized by its ability to "act in advance" of its growth.

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RESPIRATION AND CARBOHYDRATE CHANGES PRODUCED IN SWEET POTATOES BY RHIZOPUS TRITICI

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INTRODUCTION

These investigations had for their purpose to determine (1) the relative rate of respiration, as measured by the carbon dioxid (CO_2) given off by the two halves of sweet potatoes, one of which was rotted by *Rhizopus tritici* Saito, and (2) how the starch, cane sugar, and reducing sugar content of the two corresponding halves differed at the end of the experiment. It was believed that an insight into the physiological changes of the host brought about by the fungus might be obtained by experiments in which the carbohydrate content and respiration were determined simultaneously.

METHOD OF EXPERIMENTATION

RESPIRATION

The measure of the CO_2 output was determined from the corresponding halves of the same root by four separate experiments. Large, sound sweet potatoes were carefully washed, cut in two longitudinally, and pared until the two parts had the same weight. A small "well" was made in each half by means of a cork borer about 1 cm. in diameter. One half was inoculated by pouring a 24-hour growth of the organism in sweet-potato decoction (about 2 cc.) into the "well," and an equal volume of sterile water into the "well" of the control half. The two halves were then put into separate air-tight desiccators which served as respiration chambers through which CO_2-free air was drawn. The CO_2 was collected in trap bottles containing barium hydroxid ($Ba(OH)_2$) and determined as barium carbonate ($BaCO_3$). The method used for the removal of the CO_2 from the air and for its collection and subsequent determination is essentially the same as that used by the writers (*5*) [1] in the study of the respiration of fungi in nutrient solutions, to which the reader is referred for details. The respiration chambers were kept in an incubator maintained at a constant temperature of 30° C. The CO_2 given off was determined every 24 hours. By this method decay of the inoculated half was evident in 24 to 48 hours and usually complete in about 3 days. As soon as decay was complete the two halves were removed from the respiration chambers, weighed, and prepared for analysis.

[1] Reference is made by number (italic) to "Literature cited," p. 634–635.

ANALYSIS

The halves were prepared for analysis by first being run through a pulping machine. The pulp was then thoroughly mixed, and duplicate samples were transferred to tared weighing bottles for dry-weight determinations. The pulp was covered with 95 per cent alcohol and then dried to constant weight in a vacuum oven at 60° C. Another set of duplicate samples was taken from the pulp of each half for carbohydrate determinations. For this purpose 10-gm. portions of the pulp were placed in beakers containing about ½ gm. of calcium carbonate ($CaCO_3$) and were then covered with 70 per cent alcohol and heated to boiling. The contents of each beaker were then transferred to a tared thimble and extracted with 70 per cent alcohol in a Soxhlet's extraction apparatus for about seven hours. The extract was made up to 500 cc. by the addition of water, and 100 cc. were transferred to a beaker and evaporated to about 25 cc. It was then transferred to a 100-cc. sugar flask and cleared in the usual way with lead acetate. The cane sugar was hydrolyzed according to the method of Davis and Daish (*4*), and it and the reducing sugars and hydrolyzed starch were determined according to the method of Clark (*2*).

After the sugars were extracted the solid material was dried to constant weight in a vacuum oven at 60° C. The pulp was finely ground in a mortar, and a known amount was transferred to a 100-cc. volumetric flask and the starch hydrolyzed by boiling in a 5 per cent solution of hydrochloric acid (HCl) for 2½ hours with a reflux condenser. It was then neutralized by sodium hydroxide (NaOH), made up to volume, filtered, and the starch determined as reducing sugar.

The Yellow Jersey variety of sweet potatoes grown on the Potomac Flats near Arlington, Va., was used for all these investigations.

All the data were calculated to the original wet weight of the material used. The directly reducing sugar was calculated as glucose.

It might be urged that the results both of the respiration experiments and of the carbohydrate determinations were vitiated by the presence of microorganisms other than *Rhizopus tritici*. It is not unlikely that bacteria were present on the surface and that they would contribute something to the total of the CO_2 given off. Before the samples were put into the respiration chamber they were thoroughly washed but were not disinfected. The length of the respiration period was three days in all experiments except one, which continued another day longer. Cultures were made from the rotted potato at the close of the experiments, and a pure culture of *R. tritici* was obtained in every case. The writers do not believe that bacteria or fungi were present in sufficient amount to alter materially either the results of the respiration or the carbohydrate content. As a matter of fact, all respiration experiments of a sort where sterile conditions can not be employed are subject to alterations due to the presence of foreign organisms.

EXPERIMENTAL DATA

RESPIRATION

The results of all the respiration experiments expressed in grams of CO_2 given off for each 24-hour period as well as the total for the duration of the experiment are given in Table I.

TABLE I.—*CO_2 output from a decaying and a sound half of the same sweet potato at the end of each 24-hour period* [a]

Experiment No.	Sample.	First day.	Second day.	Third day.	Fourth day.	Total.
605	Control	0.6210	0.4807	0.4652	.....	1.5669
	Rotted	1.4374	4.1653	4.2861	.....	9.8888
609	Control	.9144	.9147	.9145	.....	2.7436
	Rotted	1.9583	8.5934	8.7363	.....	19.2880
631	Control	.7153	.4986	.4807	.....	1.6946
	Rotted	1.0726	5.0479	6.5600	.....	12.6805
637	Control	.3910	.4173	.3502	0.4287	1.5872
	Rotted	.9813	3.9748	4.1859	3.3626	12.5046

[a] Expressed in grams.

It will be noted that the CO_2 output at the end of the first 24 hours was considerably greater in the decaying than in the control half. This difference was further increased during the next 2 days. In the first three experiments decay was completed in 3 days and in the last one in 4 days. As shown by the last column a total of from 6.3 to 7.8 times as much CO_2 was evolved by the decaying half as by the control.

The figures presented in Table I should not be interpreted as representing exactly what happened in each individual case. In other words, an allowance should be made for variations in respiration that would normally take place between two sound halves of the same potato of equal weight. It was shown by Hasselbring and Hawkins (7) that wounding accelerates the CO_2 output of sweet potatoes. Preliminary to the experiments, the results of which are recorded in Table I, the daily evolution of CO_2 from the corresponding halves of several sound sweet potatoes was determined for the purpose of ascertaining the probable error. The two halves were held under similar conditions, and the CO_2 was collected for 24-hour periods. The greatest variation was about 11 per cent, but in most cases it was very much less. If a correction of this variation were made in each individual experiment it would not be sufficient to alter materially the ratio of the CO_2 output between the rotted and control halves.

CARBOHYDRATE CHANGES

IN LIVING POTATOES.—As soon as decay was complete the halves were removed from the respiration chambers, pulped, and prepared for the determination of starch, cane sugar, reducing sugar, and percentage of moisture. The results of these analyses are shown in Table II.

TABLE II.—*Carbohydrate content of the healthy and rotted halves at the close of the respiration period*

Experiment No.	Sample.	Moisture.	Reducing sugar as glucose.[a]	Total sugars as glucose.[a]	Cane sugar.[a]	Starch.[a]	Total loss of reducing sugar equivalent to CO_2 evolved.	Total carbohydrates as glucose not accounted for as CO_2.
		Per cent.					*Gm.*	*Gm.*
605	Control.....	67.40	3.9330	45.880	39.845	196.851	.....	.....
	Rotted.....	71.95	4.2424	10.206	5.525	165.083	6.7425	3.9699
609	Control.....	68.25	4.8709	55.308	47.915	168.541	.....	.....
	Rotted.....	73.55	8.6419	13.827	4.926	147.298	13.1509	4.4414
631	Control.....	71.30	6.4365	52.718	43.967	166.205	.....	.....
	Rotted.....	76.70	4.7507	25.440	19.655	127.260	8.6457	8.0133
637	Control.....	73.50	6.3600	70.800	61.220	166.135	.....	.....
	Rotted.....	74.60	4.9000	19.615	13.980	153.925	8.5258	5.1474

[a] Expressed in milligrams per gram of original wet weight.

The results of these analyses show that the starch, total sugars, and cane sugar are lower in the rotted than in the sound half of the potato. In the first two experiments the reducing sugars are increased and in the last two decreased. It is, of course, not possible to state from these experiments alone in what form the carbohydrates are utilized by *Rhizopus tritici*. Other investigations by the writers showed that glucose is readily utilized and cane sugar sparingly or not at all. Furthermore, in nutrient solutions this fungus will thrive better with boiled starch as a source of carbon than when cane sugar is used. On the other hand, raw starch in culture solutions (5) is hydrolyzed to some extent by *R. tritici*. Hawkins (9) found that certain species of Fusarium reduced the sucrose and reducing-sugar content of the Irish potato while the starch content was not altered and that in the peach the brownrot fungus increased the reducing sugars and the total sugars and decreased the cane sugar.

It would seem from the experiments of the writers that a fairly constant supply of reducing sugars was maintained and that the source of this supply was the cane sugar and starch.

The total amount of CO_2 evolved is shown in the last column of Table I, and in column 8 of Table II the reducing sugars equivalent to the CO_2 output is given. The total loss of the carbohydrates (starch, cane sugar, and reducing sugar) as determined from the analyses is seen to be greater than that which is accounted for by the CO_2 given off. In the last column of Table II is shown in grams the loss of carbohydrates in excess of that which can be accounted for by the CO_2 output.

What becomes of the carbohydrates can be only a matter of speculation. Hasselbring and Hawkins (7), in respiration work with sound sweet potatoes, found that the cane sugar accumulated slightly and

suggested the possibility that some of the reducing sugars may have been utilized in the manufacture of sucrose. From the results of these investigations, however, it will be necessary to search elsewhere for an explanation, since the cane sugar as well as the starch content was reduced.

In investigations of this type where microorganisms are involved, several possibilities may be advanced to account for the loss of the carbohydrates in excess of that required to satisfy the CO_2 given off. First, a portion of it undoubtedly was utilized in the construction of fungous material. No measure could be made of the fungous material formed, and although a considerable amount of mycelium was produced the actual dry weight would be small. Second, Harter and Weimer (*6*) showed that in Czapek's nutrient solution with glucose as the only available carbohydrate *Rhizopus tritici* and other fungi produced a measurable quantity of alcohol. Similar results have been obtained by other investigators with other fungi. In some cases as much as 8 per cent alcohol was produced. Probably the physiological reaction of a fungus is different when grown on a sweet potato and on an artificial medium. However, if alcohol was produced in the one case there are reasons to suspect that it might be formed also in the other. As a matter of fact, the writers have demonstrated by the use of the iodoform test the presence of alcohol in rotted sweet potatoes. Third, acid production has been demonstrated by many investigators, especially when nutrient solutions were employed. Cooley (*3*) found that *Sclerotinia cinerea* (Bon.) Schroet. formed oxalic acid when grown upon peach juice, and Hawkins (*8*) that the acid content of the rotted half of the peach, when decayed by the brownrot fungus, was higher than that in the sound half. Behrens (*1*), on the other hand, found that the acid content of the sound portion of an apple was higher than that of the rotted half. Hydrogen-ion determinations were made of the expressed juice of some of the rotted and decayed halves used in these experiments at the close of the respiration period. The results of the determinations of the samples in two experiments gave a P_H of 3.71 and 3.695 for the rotted and 5.44 and 5.032 for the sound.

That acids are produced in considerable abundance seems quite evident, and that alcohol is formed seems probable. The carbohydrates required for the manufacture of acids and alcohol, together with that utilized directly in the production of fungous material, will probably account to a large extent for the decrease in the sugars and starch which are not accounted for by the CO_2 evolved.

The foregoing results show that the cane sugar disappears at least in part from the sweet potato when decayed by *Rhizopus tritici*, indicating that it was either used directly by the fungus or converted by the host into some other compound, possibly reducing sugar. Studies of the

growth of this fungus in nutrient solutions with cane sugar as the only source of carbon have shown that this substance is used only sparingly.

This fact, together with the evidence contained in the data already submitted, indicates three possibilities: First, that the fungus can utilize to a better advantage cane sugar when other carbohydrates are present; second, that in the living potato the host under the stimulation of the fungus inverts the cane sugar; and third, that the acids produced by the fungus invert the cane sugar. The following experiments were designed to throw some light on this phase of the subject.

IN COOKED POTATOES.—Large sweet potatoes were cut longitudinally into two parts and pared to equal weight. The two halves were cut in small blocks into separate flasks and sterilized by steaming on three consecutive days. One flask was inoculated with *Rhizopus tritici* and the other one held as a control. They were both kept in an incubator held at a constant temperature of 30° C. After 15 days the starch, cane sugar, and reducing sugar of the control and inoculated flasks were determined according to the method already described. The results of these analyses are shown in Table III.

TABLE III.—*Carbohydrate content of inoculated and uninoculated cooked sweet potatoes*[a]

Experiment No.	Sample.	Reducing sugars as glucose.	Total sugars as glucose.	Cane sugar.	Starch.
670	Control	45.56	122.77	73.36	99.565
	Inoculated	0	9.02	8.569	15.525
740	Control	44.21	110.42	62.90	97.477
	Inoculated	74.025	73.685	0	14.424

[a] Calculated to milligrams per gram of original wet weight.

An inspection of Table III shows that the starch, cane sugar, and total sugar content of the inoculated material was greatly reduced, the cane sugar having disappeared entirely in experiment 740. It seems from these results that the fungus can either use cane sugar directly or convert it into some other substance before using. With respect to the reducing sugars the results of the two experiments do not parallel. In experiment 670 no reducing sugar remained in the inoculated flask, whereas in experiment 740 there was an actual increase over that of the control. This apparent discrepancy may be explained at least in part if one takes into consideration the growth of the fungus in the two cases. It was previously pointed out that glucose is an excellent source of carbon for this fungus, supporting in a nutrient solution an abundant mycelial growth. The growth of the mycelium in the first experiment was very luxuriant, about half filling a 2-liter flask, while in the second very little aerial growth took place. In view of these facts it is likely that in the former case the reducing sugars

were utilized largely in supporting an energetic respiration and in the construction of fungous material. In the second experiment with a smaller amount of fungous growth a correspondingly less amount of sugar would be utilized in supporting respiration and in the construction of fungous material. The conversion of the cane sugar and starch into reducing sugars probably took place faster than it was utilized; hence its accumulation.

Further interesting facts as regards the availability of monosaccharids and disaccharids in supporting the growth of this fungus are shown by experiments in which cane sugar and glucose alone and in combination are used as the only sources of carbon in Czapek's nutrient solution.

IN NUTRIENT SOLUTIONS.—For each experiment three flasks were prepared to contain a modification (*10*) of Czapek's nutrient solution and 1 per cent of glucose and cane sugar alone and in combination as the only source of carbon. The solutions were then inoculated, and the fungus was allowed to grow for 17 days. At the end of the growth period the mycelium was removed and the dry weight of fungous material determined. The reducing sugars and cane sugar were again determined. The loss of glucose and cane sugar as well as the dry weight of the fungous growth are shown in Table IV.

TABLE IV.—*Loss of glucose and cane sugar and amount of fungous material produced*

Experiment No.	Carbohydrates present.	Loss of dextrose.[a]	Loss of cane sugar.[a]	Dry weight of fungous material.
				Gm.
640	Glucose	52.347	0	0.3954
	Glucose and cane sugar	0	60.68	.3583
	Cane sugar	0	12.118	.2430
656	Glucose	57.99	0	.5113
	Glucose and cane sugar	0	60.09	.5158
	Cane sugar	0	0	.0853
669	Glucose	63.38	0	.5253
	Glucose and cane sugar	4.53	59.49	.5060
	Cane sugar	0	5.713	.1514

[a] Expressed in milligrams per 10 cc. of solution.

The results of these experiments show that when cane sugar is the only source of carbon a small amount of fungous material is produced. In experiment 656 there was no loss of cane sugar, and in each of the others the loss was slight. These results would seem to indicate that cane sugar when present alone in the solution is not readily utilized. On the other hand, when cane sugar and glucose are added together in the solution in equal amount, the loss of the former is relatively large but the latter remains practically constant. When, however, glucose is the only source of carbon there is a considerable reduction in the amount present at the end of the experiment.

These experiments show, then, that the cane sugar disappears when it is in combination with glucose, but that it is sparingly utilized when it is the only source of carbon. It has been shown that the growth of *Rhizopus tritici* renders the solution as well as the sweet potato more acid. In view of these facts it is probable that the increased acidity of both the decayed potato and the solutions is at least in part responsible for the inversion of the cane sugar.

SUMMARY

(1) The relative amount of CO_2 given off from the two halves of the same sweet potato, one of which was decayed with *Rhizopus tritici*, was determined. It was found that the decayed half gave off a total of from 6.3 to 7.8 times as much CO_2 as the healthy. Decay was completed in three days in all experiments except one which continued for one day longer.

(2) At the close of the experiment the starch, cane sugar, and reducing sugar were determined in the healthy and decayed halves. There was a smaller amount of starch, cane sugar, and total sugars in the decayed sample than in the healthy one, while the reducing sugars were increased in two experiments and decreased in two.

(3) The total quantity of the carbohydrates (starch, cane sugar, and reducing sugars) lost in the decayed sample, according to calculations based on the analytical data, is greater than that lost through respiration, as measured by the CO_2 given off. It seems, therefore, that a portion of the carbohydrates was utilized in other processes such as the production of fungous material, acids, alcohol, etc.

(4) When the fungus was grown on cooked potatoes (sterile) a reduction in the starch, cane sugar, and total sugar similar to that of the living potatoes took place.

(5) In Czapek's nutrient solution glucose was readily utilized when it was the only source of carbon available. When glucose and cane sugar were combined in the solution there was a reduction only in the amount of cane sugar. When cane sugar was the only available source of carbon it seemed not to be utilized by the fungus to any extent.

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WOUND-CORK FORMATION IN THE SWEET POTATO

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INTRODUCTION

The investigations reported in this paper were designed primarily to determine to what extent and under what conditions wound-cork is formed by the sweet potato and whether or not its formation serves in any way as a barrier to infection by *Rhizopus tritici* Saito.

PERIDERM FORMATION

The sections made from samples taken at the beginning of the experiments showed the condition of the tissue before any reaction to the wound stimulus occurred. The sections are made up of thin-walled cells varying somewhat in size but in the main rather large, with here and there a vascular bundle. The cells, with the exception of those comprising the vascular tissue, are more or less uniformly filled with starch. After 24 hours' exposure in a moist chamber held at room temperature (about 25° C.) a distinct change is already noticeable. A layer of cells running parallel to the exposed surface, varying from 3 to 10 deep, contains more starch than do those immediately beneath. No other change can be detected at this time, but on the second day the starch in the lower layer of cells becomes conspicuously less. About this time one or two indistinct cross walls are laid down in some of the cells in the starch-free area, so that the position to be occupied by the wound cork can be traced entirely across the section. In about 3 days the cork layer is pronounced, being from 2 to 4 cells deep, and the cell walls are considerably thickened. These changes are still more pronounced from the fourth to the sixth days. By the sixth day there is a continuous layer of cork, varying from 2 to 6 cells in thickness and lying from 2 to 12 cells beneath the wounded surface. Between the cork periderm and the layer comprising the surface cells is a third layer from 2 to 4 cells in depth which contains little or no starch and whose walls are considerably thickened and suberized, as shown by the fact that they stain deeply with iodin green. The thickening of the cell walls becomes evident about the third day. The cells immediately below the cork meristem merge into the cells containing the normal amount of starch.

It was found by the writers that the rate of cork development is retarded at the bottom of wounds made by striking sweet potatoes against the sharp edge of a wire basket. Coutant (*2*)[1] also found that the

[1] Reference is made by number (italic) to "Literature cited," p. 647.

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cork meristem in the base of a V-shaped cut was undeveloped in the 4-day-old sections. This is thought to be due to a lack of available oxygen or to the lessening of transpiration. This latter explanation is suggested by Küster (7), who showed that at least a small amount of transpiration is necessary for wound-cork formation.

EXPERIMENTAL METHODS

The sweet potatoes used in these experiments were of the Yellow Jersey variety, grown on the Potomac flats near Arlington, Va. The first samples from which the sections were cut for the determination of wound-cork formation were taken from potatoes while still in the ground. After the potatoes were put in storage samples were taken and studied at different times throughout the storage period. For the most part the potatoes were halved and blocks of tissue about 0.5 by 0.5 by 2 cm. were removed from the cut surface in such a manner that sections made across the short diameter would be perpendicular to this surface. A control sample was taken when the experiment was started, and others were taken at stated intervals of time thereafter. The samples were dropped at once into a fixer made by adding 2 cc. of commercial formalin to 100 cc. of 70 per cent alcohol. After fixing for 24 hours or longer the specimens were dehydrated with alcohol and embedded in paraffin. Sections were cut for the most part 10 μ in thickness, stained with a weak solution of iodin green in 70 per cent alcohol, ammoniacal gentian violet, or in Pianeze IIIb stain, and mounted in balsam.

In order to determine whether wound cork is produced as readily at the center of the sweet potato as it is near the vascular ring, samples were taken from both regions. A study of sections from these regions shows that although a normal cork layer is formed in both cases, it is produced more quickly in the vicinity of the vascular ring. In the latter case the cork layer laid down in two days is to all appearances equal to that formed at the center of the potato in three days. A somewhat different condition was found in the Irish potato (*9*, *12*), where periderm formation in the seed pieces is usually not so well developed in the central as in the peripheral portion.

CONDITIONS AFFECTING CORK FORMATION

Factors influencing wound-cork formation have been considered by numerous writers, who agree that a certain amount of moisture is necessary. When the cut surfaces are allowed to dry too rapidly, a desiccation and death of the surface cells soon takes place. This results in the formation of a hard surface layer beneath which cork periderm seldom forms. On the other hand, when the escape of water from the wounded surface is retarded, a cork layer usually develops beneath the injured surface. The degree of humidity present in the ordinary moist chamber is such that cork is produced by the sweet potato. Halves of sweet potatoes

held in moist chambers healed in a normal manner, while corresponding halves kept on the desk beside the moist chamber showed only a drying and death of the surface several cells in depth. It is not known why the cork layer failed to form in the latter case. However, it may possibly be due to insufficient transpiration or to the lack of oxygen. The dry and hard surface layer no doubt serves to check the interchange of moisture and oxygen between the underlying cells and the outside air. Küster (*8*) says in this connection that at least a small degree of transpiration must be possible for the exposed tissue, while he, as well as others (*6*, *10*, *1*, *4*), regards oxygen as a determining factor. Some attention has been given by the writers to the progress of healing under storage-house conditions. Experiments were conducted to show whether cork was formed under storage conditions and, if so, what effect the temperature maintained in the house at the time the potatoes were placed in storage might play. It is generally recommended that the storage house be kept at a temperature of 25° to 30° C. during the digging period and for 10 to 15 days thereafter. Sometimes no attempt is made to raise the temperature to that height until after the potatoes are all dug. This may mean the lapse of a week or more before the curing period starts.

A quantity of sweet potatoes were taken from the field to the storage house, cut into two parts longitudinally, and samples taken each day for six days. These showed a dying of the cells for a considerable depth beneath the wounded surface but no differentiation of a starch layer and no cork formation. In another experiment the potatoes were cut on the day the curing period began. Here again there was no differentiation of a starch or a cork layer but a dying of the exposed cells, which formed a hard covering over the wounded surface. No doubt either the temperature or the humidity or both were not such as to permit the normal healing process to take place.

The potatoes used in both of these experiments showed no signs of decay in the two weeks during which they were under observation. The dried surface of the wound seemed to afford an efficient barrier to infection. In order to test further the ability of a dried surface to prevent infection the following experiments were conducted.

Potatoes were taken directly from the field and placed in a dryer designed primarily for desiccating sweet potatoes and other vegetables. The potatoes were spread out thin over a wire netting, and a current of warm air was forced up among them. The quantity of potatoes used, together with the air temperature and rate of air movement, is given in Table I. The potatoes after being treated were kept in the drying room in crates until the curing period was over, after which they were moved to the sweet-potato storage house. Observations on the keeping qualities of these potatoes were made from time to time, and samples were taken for the study of the cork formation. The table shows also the

amount of decay in each crate and the quality of the potatoes which did not rot.

TABLE I.—*Effect of drying sweet potatoes in a current of warm air upon their keeping qualities*

TREATED

Quantity of potatoes.	Time of drying.	Rate of air movement in feet per minute.	Air temperature.	Percentage decayed after 3 months.	Quality of potatoes.
Pounds.			°C.		
44½	10 minute	150 to 200	46 to 49	0	Good.
41½	20 minutes	150 to 200	46 to 49	0	Do.
50	30 minutes	150 to 200	46 to 49	0	Do.
42	40 minutes	150 to 200	46 to 49	0	Do.
42½[a]	2½ hours	150	51.5	[b]99	None marketable.
46	2½ hours	150	51.5	46	Poor; few if any marketable.
43¾	3½ hours	150	51.5	63	Do.
41	4½ hours	150	51.5	22	Poor; less than one-half marketable.
43½	9 hours	190	46 to 70	59	Very poor; none marketable.
41½	9 hours	170	46 to 70	52	Do.
40	9 hours	174	46 to 70	38	Do.
42	9 hours	190	46 to 70	83	Do.

CONTROLS

40¼	None		Same as for others after treatment.	0	Good; all marketable.
42½	do			0	Do.
40¼	do			0	Do.

[a] Potatoes halved before treatment was started.

[b] In 15 days.

These results within the limits of these experiments indicate that any attempt at case hardening or of stimulating or hastening the healing of the wounds of sweet potatoes at digging time by subjecting them to a current of warm air is likely to prove detrimental rather than beneficial. The potatoes subjected to a temperature of 46° to 49° C. for 40 minutes or less were not injured appreciably, neither was their keeping quality improved. The control potatoes dug at the same time and handled in the same manner, except that they were not subjected to a current of warm air, kept equally well. On the other hand, the application of a warm air current for more than 40 minutes predisposed the potatoes to decay. It is interesting to note also that the potatoes cut in two longitudinally and then subjected to a temperature of about 51.5° for 2½ hours began to decay almost at once. Sections of some of these potatoes revealed the fact that no differentiation into a starchless layer or the formation of wound cork had taken place.

A study of the influence of temperature upon cork formation was made after the potatoes had been in storage about 2 months. Halves of potatoes from the storage house were placed in a series of chambers held at constant temperatures and humidities. Samples were taken whenever possible at intervals of 2 days for a period of 19 days. The potatoes at the higher temperatures soon decayed, so that in some cases only a few samples were obtained. Nevertheless, judging from previous work, it is thought that sufficient samples were available in every case for the purpose desired. In these tests all samples from each temperature were taken from the same half of the same potato and from a position just within the fibro-vascular ring. The temperatures and relative humidities at which these tests were carried out, as well as the results obtained, are shown in Table II. It is evident from this table that periderm formation was most rapid at a temperature of 33° C., cork production being evident in 4 days and pronounced in 8 days. The rate of cork development was about the same at 31° and 26°, producing in 11 days a layer about equal to that formed at 33° in 8 days. At 19.5° cork formation was greatly retarded, and at 11.2° no cork was formed by the fifteenth day, after which no examinations were made. Below this temperature no cork was formed within the time limit of this experiment (19 days). At 39° the potatoes decayed so rapidly that no samples were obtained after 4 days. In none of these samples was there any starchless layer formed. The walls of the cells just outside of the region where the cork layer probably would have developed if conditions had been favorable were usually somewhat thickened and suberized. The surface and subjacent cells were always dead and brown to a varying depth, depending upon the conditions of the environment.

TABLE II.—*Effect of temperature upon periderm formation*

Temperature.	Relative humidity.	Condition after healing—					
		4 days.	6 days.	8 days.	11 days.	15 days.	19 days.
°C							
44	95	(*)	(*)	.....	.....	.....	.....
39	96	(*)	.....	.....	.....	.....	.....
33	96	(†)	.....	(‡)	.....	.....	.....
31	96	.....	.....	(†)	(‡)	.....	.....
26	96	.....	.....	(†)	(‡)	.....	.....
19.5	95	.....	.....	.....	(†)	.....	.....
11.2	97	.....	.....	.....	.....	(*)	.....
8.5	96	.....	.....	.....	.....	(*)	.....
6.8	96	.....	.....	.....	(*)	.....	.....
1	95	.....	.....	.....	.....	.....	(*)

* No cork layer. † First indications of cork layer. ‡ Cork layer well developed.

This experiment shows that a protective cork layer will form at temperatures between 26° and 33° C., while below 26° the process is greatly retarded. As stated above, sweet potatoes are cured for from

10 days to 2 weeks when first placed in storage by subjecting them to a temperature of about 25° to 30°. This is likewise the most favorable temperature for periderm formation. Experiments previously recorded showed that in those particular instances no cork was formed preceding or during the curing period. In all probability the relative humidity was the inhibiting factor. Similar experiments conducted at intervals of about 2 months throughout the season showed that at no time were the potatoes able to form a cork layer over a cut surface under storage-house conditions. However, controls held in moist chambers in the laboratory at the same time never failed to do so.

Although no periderm formed beneath cut surfaces under storage-house conditions, a cork layer was laid down just beneath dead rootlets. Likewise a similar layer of cells was laid down just beneath the wound made by breaking the potato from the roots. In the former case the dead rootlets probably served to prevent a too rapid drying of the wound, and a cork layer was formed. Likewise the deposit of the latex over the wounded end of a freshly dug sweet potato probably forms a protection against the escape of moisture, which may explain in part at least the formation of a cork layer under such conditions.

Sweet potatoes which have sprouted either in the hotbed or in the storage house have the power to form a cork layer when placed under suitable conditions. Some sweet potatoes with soft and flabby ends were placed in moist chambers in an attempt to learn whether they would form a cork layer. In most cases, however, the exposed tissues at the dried end soon turned brown and began to decay. This would seem to indicate that sweet potatoes lose their power to form a protective covering over their wounds when they once become badly shriveled, the degree of drying being the deciding factor. It has been demonstrated also (*12*) that sprouted Irish potatoes form a new cork over wounds, but that drying of the tissues, which usually accompanies germination when potatoes are stored in a warm room for a long time, retarded it.

The skin of the sweet potato, as well as that of the Irish potato (*11*), serves as an effective protection against the death of the tissues beneath by desiccation and from the attack of microorganisms. When this is broken the exposed cells would soon die were it not for their power to form some sort of protective covering. Incidentally this protective layer may possibly, at least to some extent, serve to prevent infection by microorganisms.

EFFICIENCY OF THE WOUND CORK IN PREVENTING INFECTION

The writers have nearly always failed to produce infection in sweet potatoes by Rhizopus by smearing spores and hyphae over a freshly cut surface. Nevertheless, wounded potatoes subjected to certain condi-

tions will almost invariably decay by this organism. It was thought that perhaps these studies might show some correlation between the cork formation and the susceptibility of the potatoes to decay. Consequently, experiments were designed to test the resistance of the potato to infection through wounds which had been allowed to heal for different lengths of time. In these experiments potatoes were cut in two parts and placed in a moist chamber. After they had healed for a definite length of time a fresh cut was made in one half, which was to serve as a control. Both halves were then inoculated, and the results were noted. The "well" method described by Harter, Weimer, and Adams (*3*) was used in most of the experiments described below. In these cases a hole was made into the cut half of the potato with a cork borer at the time the experiment was started and a similar hole was made in the control half on the day the inoculations were made. In other cases the method was slightly modified, in that a hole was made in the cut surface of the potato with a knife and a similar hole in the control later. In both of these cases the inoculations were made by pouring into the cavities a 24-hour-old culture of *Rhizopus tritici* in about 5 cc. of sweet-potato decoction. About half of the mycelial growth formed by this organism growing on sweet-potato decoction in a 150-cc. Erlenmeyer flask was placed in some instances upon the flat cut surface of one half of a potato which had healed for a certain number of days and the remainder on the other half, the control, from which a slice about ½ cm. thick parallel to the wounded surface was first removed.

In general, the results shown in Table III indicate that the cork layer does possess some power to prevent infection by Rhizopus, but in the majority of cases under the conditions of these experiments infection is only retarded. The method used is subject to criticism in that the test was an extreme one, since a large amount of very active hyphae and enzym was placed in contact with the healed surfaces. Such conditions would never be duplicated in nature, and probably the cork layer would never be required to withstand such a rigid test under natural conditions. However, as pointed out above, attempts to infect even a freshly cut potato with dry spores or hyphae almost always fail. It was necessary, therefore, to use some method whereby infection of the control could be obtained with certainty.

The last column in the table shows those instances where a cork layer was actually demonstrated to be present by free-hand sections.

It was pointed out above that a cork layer did not form so readily about the base of a wound made by striking a potato against a dull instrument. Although no attempt was made to determine the rate of cork formation about the base of the "well," it seems reasonable to suspect that it might not develop at a uniform rate in all places. In that case these tests of course would not necessarily indicate the true value of healing as it might

occur over a flat surface. This last criticism could not hold, however, in the cases where a piece of fungous felt was placed upon the cut surface. Here infection was retarded but not inhibited.

TABLE III.—*Effect of healing upon decay of sweet potatoes*

Date.	Number of potatoes.	Method of inoculation.	Time healed.	Results.			Presence of cork layer demonstrated.
				Control halves decayed.	Healed halves decayed.	Effect of healing.	
Nov. 17, 1919..	1	Felt placed on surface.	24 hours.	1	1	None.....	(a)
Do.....	1	.....do.....	48 hours.	1	1	Retarding decay.	(a)
Do.....	1	.....do.....	3 days...	1	1	.....do.....	Cork layer starting.
Do.....	1	.....do.....	4 days...	1	1	.....do.....	Distinct cork layer.
Do.....	1	.....do.....	7 days...	1	1	.....do.....	Do.
Do.....	1	.....do.....	11 days..	1	0	Inhibited decay.	Do.
Dec. 3, 1919....	9	Well method.	6 days...	9	9	None.....	(a)
Dec. 5, 1919....	2	.....do.....	24 hours.	2	0	Inhibited decay.	(a)
Do.....	1	.....do.....	4 days...	1	0	.....do.....	(a)
Dec. 12, 1919...	10	.....do.....	24 hours.	10	10	None.....	(a)
Jan. 22, 1920...	4	.....do.....	6 days...	4	3	Inhibited decay in 1 case.	(a)
June 17, 1920...	12	.....do.....	4 days...	12	12	Marked retarding of decay.	Distinct cork layer.
Mar. 20, 1920....	9	.....do.....	...do.....	9	9	None.....	(a)

a Not examined or no cork layer found.

In order to study the effect of healing upon infection when cut sweet potatoes were subjected to conditions more nearly approximating those to which they would normally be exposed, the following experiment was conducted. About ¾ bushel of medium-sized potatoes were washed with soap and water, halved, and then held for 6 days at a temperature of 30° to 32° C. and a relative humidity of about 93 as registered by a standardized Lambrecht's polymeter. The potatoes were then divided into four lots. The healed surfaces of two lots were smeared with a heavy suspension of Rhizopus spores in tap water, using a soft camel's-hair brush. The other two lots (controls) were treated in exactly the same way, except that the healed surfaces were cut away to a depth about 5 mm. before the spores were applied. One lot from each of these two sets was placed in an incubator maintained at a temperature of 25° and a relative humidity of 96. The other two lots were placed

in the sweet-potato storage house at a temperature of about 20° to 25°. Some potatoes were taken from the storage house at the time the experiment was set up, cut in halves, and the cut surfaces also smeared with the spore suspension. Table IV shows the results obtained as indicated by the final observations taken after 18 days.

TABLE IV.—*Efficiency of the healed surface in preventing decay of sweet potatoes by Rhizopus*

Treatment.	In incubator.			In storage house.		
	Number of halves decayed.	Number of halves healthy.	Percentage decayed.	Number of halves decayed.	Number of halves healthy.	Percentage decayed.
Healed	0	35	0	1	54	1.8
Healed surface removed	9	5	64	4	12	25
Freshly cut potatoes from storage house	8	6	57	16	4	80

The data contained in Table IV show that under the conditions of this experiment the healed surface formed a very efficient barrier to infection. In the incubator none of the healed potatoes decayed. On the other hand, 64 and 57 per cent, respectively, of those from which the healed surface had been removed and the potatoes which were freshly cut decayed. Somewhat similar results were obtained with potatoes held in the storage house. However, in this instance one healed specimen decayed, while 25 and 80 per cent, respectively, of the unhealed halves rotted. Since the conditions of this experiment more nearly approach those to which wounded potatoes are normally subjected, there seems to be little doubt that the healed surface forms a quite effective barrier against the attack of microorganisms. This is further substantiated by other experiments which are recorded below.

In one experiment 11 potatoes which were injured at digging time and had formed a hard protective covering over the wounds were used. A Van Tieghem cell was sealed upon the injured surface with vaseline. A 24-hour-old culture of *Rhizopus tritici* in sweet-potato decoction was poured into this cell and the top covered with a cover slip to prevent evaporation. The potatoes were held in a moist chamber at room temperature. In no case did any rot occur.

A similar experiment was conducted in which the potatoes used had been cut and held in a constant-temperature chamber at a high relative humidity for about 3 weeks. Sections through the cut surface did not show a definite cork layer, but a subserization of some of the cell walls had taken place, as was denoted by their reaction to iodin green. Four halves were used in this experiment, and in no case did any rot take place. Four other potatoes from this same lot were inoculated in the side opposite the cut surface by the ordinary well method. These were

all decayed in 5 days. The fungus, however, was not able to soften the hardened layer of tissue over the healed surface. The inability of *Rhizopus tritici* to break down this protective wound covering was demonstrated further by placing sections of this tissue in sweet-potato decoction upon which this fungus had grown for 3 days. Freshly cut sweet-potato blocks used as controls were entirely softened in less than 48 hours, whereas the tissue over the wound was not affected after 4 days.

The length of time necessary to form an impenetrable barrier over a wounded surface no doubt varies with the conditions. Data presented in Table III show that under the conditions realized in some experiments a considerable retarding effect was exhibited after 48 hours, while in other cases there seemed to be no delay or inhibition of decay after healing for 6 days.

It has been demonstrated (*1*) that a cut surface of the Irish potato healed to such an extent in 12 hours that a virulent culture of *Bacillus phytophthorus* Appel was unable to penetrate the tissue; furthermore (*5*), that wounded potatoes when inoculated 3 hours after being placed in an incubator at 30° C. failed to become infected, a protective covering having already formed.

It has also been found (*4*) that the wound cork is very effective in preventing infection of the sweet potato by *Mucor stolonifer* Ehrb. Similar results were obtained by other workers with these and other crops.

The fact that the presence of a definite cork layer could not be demonstrated in some cases where decay was inhibited or retarded would seem to indicate that this layer is not alone responsible for the protection of the wounds. Certainly no cork layer could have been laid down in the instances mentioned above, where infection of the Irish potato was inhibited by 12 hours' healing and in the case where it failed to become infected through a surface healed for only 3 hours. No doubt the suberization of the surface cell walls is a factor in preventing the entrance of microorganisms, and even the drying out of the injured cells may exert some retarding or inhibiting action against decay-producing organisms.

SUMMARY

(1) Sweet potatoes have the ability to form a cork layer over wounds when the environmental conditions are favorable.

(2) The production of a cork layer is preceded by the formation of a layer of starch-free cells, usually 3 to 10 cells deep, beneath the injured surface. Cross walls begin to appear from the second to the third day, and by the fourth to the sixth day a distinct layer of cork cells forms a covering over the wound.

(3) Temperature and humidity are important factors in regulating the formation of cork in the sweet potato. This process took place more

rapidly at 33° C. than at any other temperature tried. However, cork cells were formed at temperatures varying from 19.5° to 33°. A relative humidity of from 95 to 100 is favorable for cork production.

(4) No well-developed cork layer was produced over wounds under the conditions existing in the sweet-potato storage house. However, a dry, hard surface covering was formed through which infection by artificial means could not be obtained.

(5) The results of numerous experiments indicate that the healed surface of a wounded sweet potato forms a fairly efficient barrier against infection by microorganisms.

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TWO SCLEROTIUM DISEASES OF RICE

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SEEDLING-BLIGHT CAUSED BY SCLEROTIUM ROLFSII SACC.

In the spring of 1919 the Office of Cereal Investigations undertook a study of the rice diseases of the United States with field headquarters for the work at the Rice Experiment Station, Crowley, La., conducted cooperatively with the Louisiana Agricultural Experiment Station. On June 12 of the same year Mr. J. Mitchell Jenkins, superintendent of the Rice Experiment Station, called the writer's attention to a seedling-blight occurring in Honduras rice and resembling maggot injury. After close examination of diseased plants, no signs of the root maggot could be found, but the constant association of mycelial fibers and brown sclerotia with diseased parts was good evidence that the disease was of fungal origin. As the season advanced, the disease was found occurring to a greater or less extent in the various sowings on the Station farm. Varieties of both the long-grain and short-grain types of rice were affected. In the summer of 1920, the disease was equally as abundant on the experiment farm. It was found also on other farms near Crowley but was not so abundant. Later in the summer of 1920, while the author was at Gueydan, La., studying the "straighthead" disease of rice, the farmers seemed to be more interested in a seedling-blight which had been causing considerable damage in that vicinity. According to their descriptions of the disease it was identical with the seedling-blight at Crowley, although it was too late to obtain specimens for identification. Reinking [1] reports the occurrence of a Sclerotium disease of rice in the Philippine Islands. He states that damage is caused in the seed bed and in rice near maturity, where the stems are attacked and sterile heads result. Reinking did not attempt to place the fungus specifically, but cultures of the Philippine form obtained from Mr. G. O. Ocfemia, a Philippine student in plant pathology at the University of Wisconsin, apparently are identical morphologically with the form in Louisiana.

SYMPTOMS

The appearance of small areas of blighted or dead seedlings is the first noticeable sign of the disease in the field. (Pl. 122, A.) These areas tend to follow the drill rows, and a part or all of the seedlings may be

[1] Reinking, Otto A. Philippine economic-plant diseases. *In* Philippine Jour. Sci. Sect. A, v. 13, no. 5, p. 228. 1918.

dead for several feet or even several yards. The disease may follow a row or extend across two or more rows, which are about 8 inches apart. The dead plants have a dark, moldy appearance, which indicates slow death and the presence of various saprophytic fungi. Alternaria and Helminthosporium are commonly present on these dead plants. Plants which are killed by maggots are usually lighter in color, due to the rapid drying after the stems are severed from the roots. The base of the stem and the roots of diseased plants are dark in color and often have a frosty appearance, due to the presence of wefts of the mycelium of the causal fungus. Brown sclerotia may be found attached to the roots or the base of the stem of diseased plants. Plants attacked by this fungus are not so easily removed by pulling as are plants severed at the base by root maggots, although the roots are almost completely decayed by the time the plants are dead. Infected plants first show a stunted condition accompanied by yellowing and withering of leaves, and a high percentage of the plants may show a distinct lack of chlorophyll or "pseudo-albino" condition, which may appear as white stripes in the leaves or as almost totally white leaves. Part or all of the leaves on a plant may show this condition. Rice seedlings weakened by other root troubles in some cases may show this same lack of chlorophyll. Plants badly affected die slowly, the tips and margins of leaves dying first and thus affording a medium of early attack by saprophytic forms. Plants that survive until the irrigation water is applied, if not too nearly dead, usually recover, due to the fact that water immediately checks the development of the fungus.

IMPORTANCE OF THE DISEASE

The distribution of the disease is not known, and consequently no estimate of damage can be given. In localities where it occurs, however, considerable losses may result. Early sown rice seems more subject to attack by the fungus than that sown later. This probably is due to the fact that seed sown later germinates more readily and the plants grow more vigorously, thus giving the fungus less chance to overcome them before the irrigation period. The quantity of fungus material may be reduced somewhat because of the decrease of organic matter in the soil which serves as food for it. The fact that a large percentage of the seedlings may be destroyed before emerging from the soil makes the disease more important than might be suspected from the dead seedlings seen in the field. By digging into the soil, seedlings in various stages of development are found to have been destroyed.

Still more importance was attached to this disease when an apparently identical fungus was found growing in abundance on soybean plants on the station farm where the soybean is being considered as a desirable crop to rotate with rice. If these forms proved to be identical there would be considerable danger of disseminating the fungus with soybean seed.

CAUSAL ORGANISM

Small, brown, spherical sclerotia were found constantly associated with the decayed roots of blighted seedlings. The coarse, white mycelium typical of *Sclerotium rolfsii* was present at the base of the stems, on the diseased roots, and in the soil immediately surrounding the roots. Fragments from freshly diseased tissues, plated on sterile agar, gave pure cultures of *S. rolfsii*, as did the sclerotia which were surface-sterilized and sown on sterile media. The organism grows vigorously and produces sclerotia abundantly on boiled rice. In culture it appears slightly different morphologically from the forms occurring on soybean, wheat, and tall oat grass (*Arrhenatherum elatius* (L.) Beauv.), but more nearly like the form from wheat. The difference, however, in any case is very slight. The sclerotia of the form on rice are more uniformly spherical, the mycelium is not so coarse in appearance, and there is also a physiological difference brought out later in comparing the ability of these forms to parasitize rice. There is not enough difference, however, to warrant their separation as distinct species.

The rice fungus is not a vigorous parasite. It attacks germinating seed and young seedlings before the root system is sufficiently established to combat the invasion. It is very destructive to germinating seed but acts as a slow parasite on seedlings that have emerged, gradually destroying the tissues of the roots from the surface inward (Pl. 124) until the seedlings are dead. It kills seedlings readily when grown in tubes on nutrient agar (Pl. 123). If there is an abundance of the organism in the soil the stand of plants may be greatly reduced (Pl. 122, B). On soil inoculated by sowing sclerotia with the seed the stand was reduced as much as 50 per cent in some cases. Comparisons were made with the various strains of *Sclerotium rolfsii* mentioned above—namely, from rice, soybean, wheat, and tall oat grass. The results of these experiments, given in Tables I and II, show that the forms from rice and wheat are equally parasitic to rice, while the forms from soybean and tall oat grass did but little damage to rice. This does not mean necessarily that all cultures from the soybean and tall oat grass would be such weak parasites to rice. They also might become more parasitic after a period of adaptation accomplished through contact with the rice plant.

For experimental purposes, large galvanized cans 16 inches square and 15 inches deep were used. These were filled with about 10 inches of soil from a field where rice was never grown, obtained in such a way as to avoid contamination as much as possible. Each can had a drain pipe at the bottom for the purpose of regulating the water supply. The cans were arranged in series of 10, and soil was banked against them on the sunny side to prevent overheating and too rapid evaporation (Pl. 122, B). The soil was inoculated by sowing the fungus sclerotia with the rice seed. Honduras rice was used for these experiments, and 100 seeds were sown in each can.

TABLE I.—*Results of germination of Honduras rice in soil inoculated with Sclerotium rolfsii from rice, soybean, tall oat grass, and wheat; sowings made on May 4, 1920, and counts taken May 22*

Can No.	Source of fungus.	Treatment.	Percentage of germination.[a]	Number of plants chlorotic.	Number of plants dead before irrigation.
1	Rice	Inoculated	25	3	0
2	do	Control	**71**	0	0
3	do	Inoculated	40	10	4
4	do	Control	**55**	0	0
5	do	Inoculated	37	5	3
6	do	do	28	5	2
7	do	do	24	4	1
8	do	do	33	3	2
9	do	do	33	7	5
10	do	do	44	10	4
1	Tall oat grass	do	79	2	1
2	do	do	71	3	0
3	do	do	70	7	0
4	do	do	63	2	0
5	do	do	78	8	0
6	do	do	58	3	1
7	do	do	66	3	0
8	do	do	63	0	1
9	do	Control	**73**	1	0
10	do	do	**71**	0	1
1	Soybean	Inoculated	57	0	1
2	do	do	55	0	0
3	do	do	59	1	4
4	do	do	48	3	0
5	do	do	62	1	1
6	do	do	63	1	0
7	do	do	58	0	0
8	do	do	69	0	0
9	do	Control	**71**	0	0
10	do	do	**72**	0	0
1	Wheat	Inoculated	34	5	0
2	do	do	36	10	0
3	do	do	34	4	0
4	do	do	34	3	1
5	do	do	23	4	0
6	do	do	18	3	1
7	do	do	35	5	0
8	do	do	25	9	1
9	do	Control	**74**	0	0
10	do	do	**59**	0	0

[a] Controls are given in boldface type.

Table I shows that the percentage of rice germination was considerably reduced where the soil was inoculated with *Sclerotium rolfsii* from rice and wheat, while strains of the organism from soybean and tall oat had but little effect on germination, although some of the seedlings were diseased. The average gemination in soil inoculated with the rice fungus was 33 per cent, as compared with 63 per cent in uninoculated soil. There was an even more striking difference where the soil was inocu-

lated with sclerotia from the wheat fungus. Here the percentage of germination in inoculated soil was only 29.9, as compared with 66.3 in uninoculated soil. There was a germination of 59 and 71.5 per cent, respectively, in soil inoculated with the soybean fungus and in uninoculated soil, and a germination of 68.5 and 72 per cent, respectively, in inoculated and uninoculated soil where the tall oat grass form was used. The difference in the case of the tall oat grass form could easily have been a variation in individual controls, as some of them ran as low as 63 per cent. There was a noticeable reduction, however, where the soybean fungus was used as the inoculum. Diseased plants in these experiments continued dying until the irrigation water was applied, after which time all plants recovered, if not too severely damaged. The high percentage of chlorotic or pseudo-albino plants was very striking where the disease was present. A high percentage of these chlorotic seedlings died before irrigation.

TABLE II.—*Results of germination of Honduras rice in soil resown on June 29, 1920, after removal of a crop 7 weeks old*[a]

Can No.	Source of fungus.	Treatment.	Percentage of germination.	Number of plants chlorotic.	Number of plants dead before irrigation.
3	Rice	Inoculated	67	0	2
4	do	do	59	0	0
5	do	Reinoculated	30	1	5
6	do	Inoculated	81	0	0
7	do	do	64	0	2
8	do	do	75	0	0
9	do	Control	74	0	0
3	Tall oat grass	Inoculated	75	0	0
4	do	do	61	0	0
5	do	Reinoculated	51	12	4
6	do	Inoculated	75	0	0
7	do	do	74	0	0
8	do	do	67	0	0
9	do	Control	74	0	[b] 1
3	Soy bean	Inoculated	74	0	0
4	do	do	75	0	0
5	do	Reinoculated	70	7	2
6	do	Inoculated	64	0	0
7	do	do	74	0	1
8	do	do	59	0	0
9	do	Control	70	0	0
3	Wheat	Inoculated	60	0	1
4	do	do	80	0	3
5	do	Reinoculated	41	5	8
6	do	Inoculated	61	0	0
7	do	do	76	0	2
8	do	do	64	0	0
9	do	Control	68	0	0

[a] At the time of the first seeding the soil was inoculated with *Sclerotium rolfsii* from rice, soybean, tall oat grass, and wheat. Can No. 5 in each series was reinoculated.
[b] Worm injury.

Two inoculated cans and one control can from each series were irrigated at a depth of 4 to 5 inches 20 days after planting, and none of the plants died after the irrigation water was applied. Plants which showed signs of the disease revived rapidly after the application of the water. About 7 weeks after sowing the plants were removed from the remaining seven cans in each series, and the cans were resown. The Honduras variety of rice was used, as in previous experiments, and 100 seeds were sown in each can. Can No. 5 in each series was reinoculated with fresh cultures of the same organisms used with the previous sowings. Results of these experiments are given in Table II.

The results given in Table II indicate that the fungus is practically exhausted after eight weeks in the soil where rice is grown. Soil that was reinoculated (can No. 5) in each case showed the same proportionate decrease in germination as was shown in Table I.

ENDURANCE OF THE FUNGUS

The fungus remains in the sclerotial stage during seasons that are unfavorable to growth. The mycelium will live for considerable lengths of time in the infected plant tissues and in the soil where there is sufficient organic matter to furnish food. Sclerotia kept in a dry condition for nine months germinated readily when placed under proper conditions of moisture and temperature. On May 28, 1920, sclerotia of the fungus were placed in small vials with cheesecloth over the top and immersed in water in the laboratory and in the field where rice was irrigated. On September 10 these sclerotia were almost 100 per cent viable. They were still viable on November 4. Under field conditions where the disease occurred the fungus lived through the period of irrigation and started developing on the roots and stems of plants after the fields were drained.

DISSEMINATION

The sclerotia float on the surface of water like cork and may be carried easily by irrigation water. The fungus also grows vigorously as a saprophyte and may be carried about on old rice straw or in hay or other plant material that is moved from fields where it occurs.

CONTROL MEASURES

Thorough cultivation hastens the decay of organic matter in the soil and also favors the germination of the sclerotia of the fungus. If infested soil is plowed early, allowed to stand for a few weeks, and then disked or well harrowed before seeding, the organic matter will have a chance to decay and the fungus will be more or less exhausted. Rice sown later than the average germinates readily and grows vigorously, thus giving the fungus less chance for attacking it. This is especially true where the soil is well prepared and a good seed bed is made. Seed sown before the soil has become thoroughly warm does not germinate promptly, and the growth of the seedlings is slow. Seedlings in such a

weakened or slow-growing condition are very susceptible to attacks by parasitic organisms, a fact which probably accounts for the higher percentage of the disease in early sowings.

The fungus requires considerable air for its growth and consequently does more damage to rice seedlings when the soil is rather dry. Reinking [1] states that—

> When there is a lack of water in seed beds, the disease appears to be at its worst. Seed beds should be kept flooded.

When the land is irrigated the growth of the fungus is checked and no further damage is caused, while all plants recover that are not too nearly dead. If the disease begins to develop, the field should be irrigated as soon as possible. Observations, both in the field and in sowings made in inoculated soil, reveal the fact that the disease is completely checked by water. If the soil of a field is known to be infested, a much better stand may be obtained by irrigating the land sufficiently to wet it thoroughly at the time the seed is germinating. This would tend to hold the fungus in check at the most critical stage in the seedling growth, as shown in Tables I and II. These experiments showed that the greatest injury was done before the seedlings emerged from the soil.

To control the spread of the disease the rice grower should prevent irrigation water from flowing from infested fields to clean fields and avoid taking rice straw or hay from fields where the disease occurs and putting it on other soil, as the organism may be growing saprophytically on such dead material. Although the fungus from the soybean proved much less parasitic on rice than did the rice form, it is still possible for the rice fungus to be carried on the the soybean plants. It may also be carried as a saprophyte, and it is advisable to select clean bean seed, especially if it is to be sown on uninfested land.

STEMROT CAUSED BY SCLEROTIUM ORYZAE CATT.

In the rice fields at Crowley, La., in the spring of 1919, an abundance of small, black sclerotia were found in the tissues and on the surface of old rice plants and stubble of the previous year. The same condition was noted again in the spring of 1920, at which time the organism was obtained in pure culture from the sclerotia. The fungus was then thought to be of an entirely saprophytic nature. On August 5, 1920, considerable lodging was noted in a field of Early Prolific rice which was being harvested near Crowley. The affected plants were more or less collapsed, and the panicles were generally poorly filled and light. Near the ground, two or three nodes from the base, the stems appeared darker, and the internode where the irrigation water stood was found to be almost completely destroyed (Pl. 125). Only the thin epidermal layer remained intact, and the stem often was completely collapsed at this point. In the cavity of the diseased portion of the stem and in the diseased tissues

[1] Reinking, Otto A. Philippine economic-plant diseases. *In* Philippine Jour. Sci. Sect. A, v. 13, no. 5, p. 228. 1918.

a weft of fine, white mycelium and numerous small spherical sclerotia could be seen (Pl. 126). Sclerotia were noted also in the sheath tissues. Apparently the sheath is the first part of the plant to be attacked.

Indications are that the sclerotia float on the water and thus come in contact with the leaf sheath, where they germinate and penetrate the tissues, causing dark brown areas. These lower sheaths die when submerged in the irrigation water, and the fungus lives more or less as a saprophyte in the dead tissues and finally attacks the stems after the plants are older. The most severe damage is caused at the time the vitality of the stem is declining and the panicles are filling. The plants are weakened, and the resulting panicles are poorly filled and light.

The fungus is easily cultured from sclerotia by dipping them in alcohol and then in mercuric chlorid (1 to 1,000) for five minutes, washing in sterile water, and plating on agar. Fragment cultures from invaded tissues also are successful. The organism grows vigorously on boiled rice and produces an abundance of the small black sclerotia. The mycelium, which is normally white, becomes a dark smoky color at the surface of the medium. The hyphae are 3 to 5 microns in diameter, septate, and profusely branched. Dry, mature sclerotia from the diseased stems range in size from 220 to 270 microns in diameter, averaging about 250 microns. They are black, and rather uniformly spherical, with a smooth surface.

Cattaneo [1] described the organism in Italy in 1879 as follows:

> Sclerotia black, 1/10 mm. in diameter, glistening, arising from a slendor white mycelium.

The measurements of sclerotia given by Cattaneo are smaller than sclerotial measurements of the form found in Louisiana, but in all probability his measurements were roughly made. Shaw [2] gives a range of measurements of the sclerotia of *Sclerotium oryzae* which easily covers the dimensions of the American form. Shaw measured sclerotia from cultures, however, and this probably accounts for the wider variation in size. His description of the fungus on glucose agar is as follows:

> The sclerotia are at first visible as minute circular spots of a grayish color; subsequently they become black and shiny, exactly resembling those found in the rice plant. The hyphae are of the usual type, the cells being about 4 to 6 microns broad and 150 to 350 microns long. They contain numerous oil globules and frequently branch. A tranverse septum occurs at the point of origin of a branch and not some distance from it. The sclerotia are roughly circular and vary in diameter from 150 to 500 microns. They arise from a plexus of interlacing hyphae which continue to branch and intertwine until a small, spherical mass is formed. For a time the young sclerotium increases in size by the adhesion of fresh branches to the periphery; ultimately the cell walls turn black and all further growth ceases.

Symptoms of the disease given by the eastern writers are identical with the symptoms of diseased plants in Louisiana with the exception of tiller-

[1] CATTANEO, Achille. SULLO SCLEROTIUM ORYZAE, NUOVO PARASSITA VEGETALE . . . *In* Arch. Lab. Bot. Crittogamico R. Univ. Pavia, v. 2/3, p. 75–83, pl. 7. 1879. Bibliografia del genere Sclerotium, p. 81–83.

[2] SHAW, F. J. F. A SCLEROTIAL DISEASE OF RICE. *In* India Dept. Agr. Mem. Bot. ser., v. 6, no. 2, p. 11–23, 3 pl. 1913. Bibliography, p. 21–22.

ing, mentioned by Shaw [1] and Butler [2] in India. This condition does not occur in this country to any noticeable extent and is not mentioned by Miyake [3] in Japan. This perhaps is due to earlier attacks by the fungus in India, which cause the injured plant to produce tillers. All other evidence indicates that the disease in the various countries is caused by *Sclerotium oryzae*. The fungus was probably introduced into America with seed rice from some of the oriental countries.

Honduras rice plants were inoculated by inserting mycelium of the fungus into needle punctures in the stem tissues near the water surface. Within 11 days there were noticeable lesions, and one plant had fallen from stemrot. One month after inoculation all plants were diseased, and sclerotia were present in the diseased tissues of some of them. Shaw [1] reports 70 to 80 per cent of fatalities to rice seedlings grown on nutrient agar and inoculated with the fungus.

In 1920, the disease did considerable damage to early rice in the vicinity of Crowley, La., where it was first noted in this country. Early Prolific rice is especially susceptible to its attack, but all varieties were more or less affected. The disease was found also at Elton, La., where it did noticeable damage in 1920. Reports of its occurrence in Arkansas have been received, but specimens have not been obtained for identification. Cattaneo [4] states that the disease was destructive in Italy as early as 1879. Miyake [3] reports its occurrence in Japan in 1910 and says great damage is caused to the rice crop where diseased. Shaw [1] and Butler [2] in 1913 report considerable damage in various localities in India due to this disease.

During the winter and other periods unfavorable to growth the fungus survives in the sclerotial stage. The sclerotia from old rice straw and stubble germinate readily in the spring. These sclerotia can be carried easily from field to field by the irrigation water on which they float. Straw removed from the diseased areas also will carry the fungus.

The Japanese varieties apparently are less susceptible to the disease than are the long-grain rices. Early Prolific seems to be the most susceptible of all. There is a possibility of developing a resistant variety by selecting plants which withstand the attacks of the fungus. All diseased plants should be carefully removed from plots where seed rice is to be obtained. Where the soil is infested with the fungus the more resistant varieties should be grown in preference to varieties that are known to be highly susceptible.

[1] Shaw, F. J. F. op. cit.

[2] Butler, E. J. diseases of rice. *In* Agr. Research Inst. Pusa Bul. 34, p. 34–36. 1913.

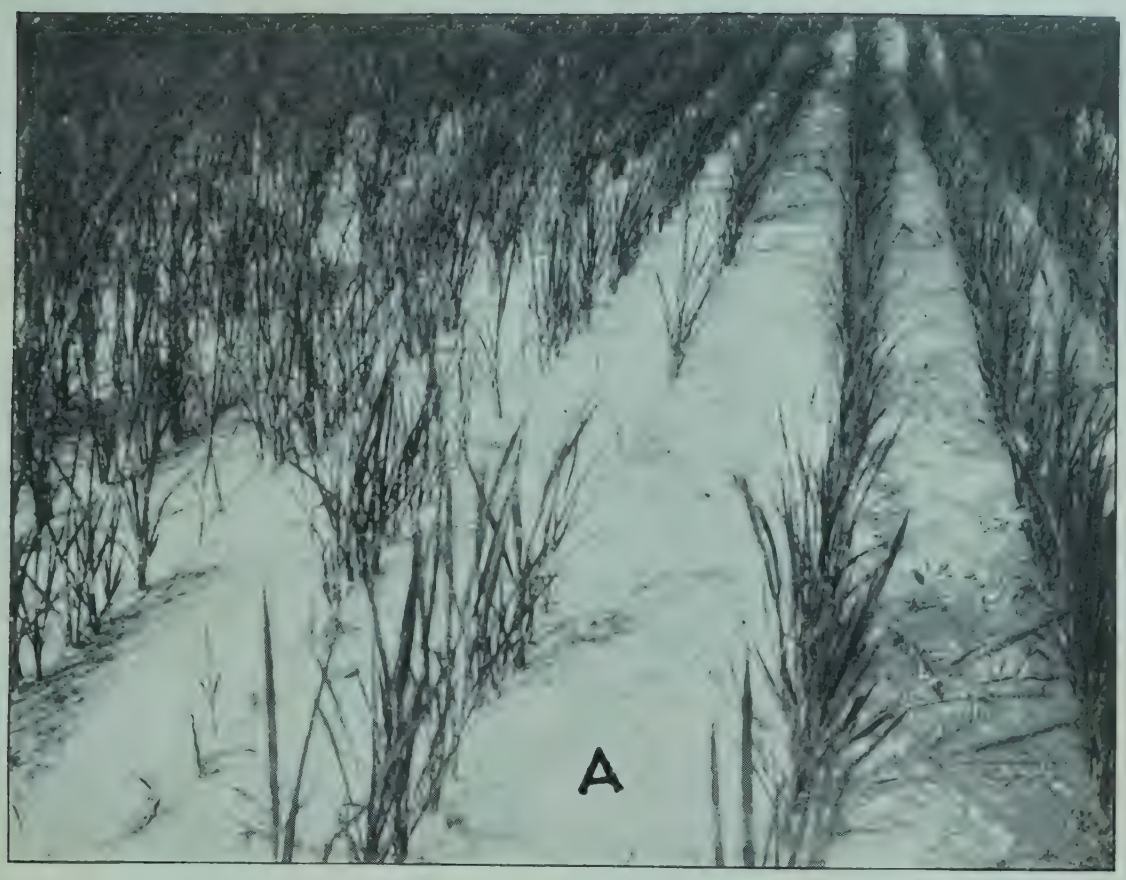
[3] Miyake, Ichiro. studien über die pilze der reispflanze in japan. *In* Jour. Col. Agr. Imp. Univ. Tokyo, v. 2, no. 4, p. 264. 1910.

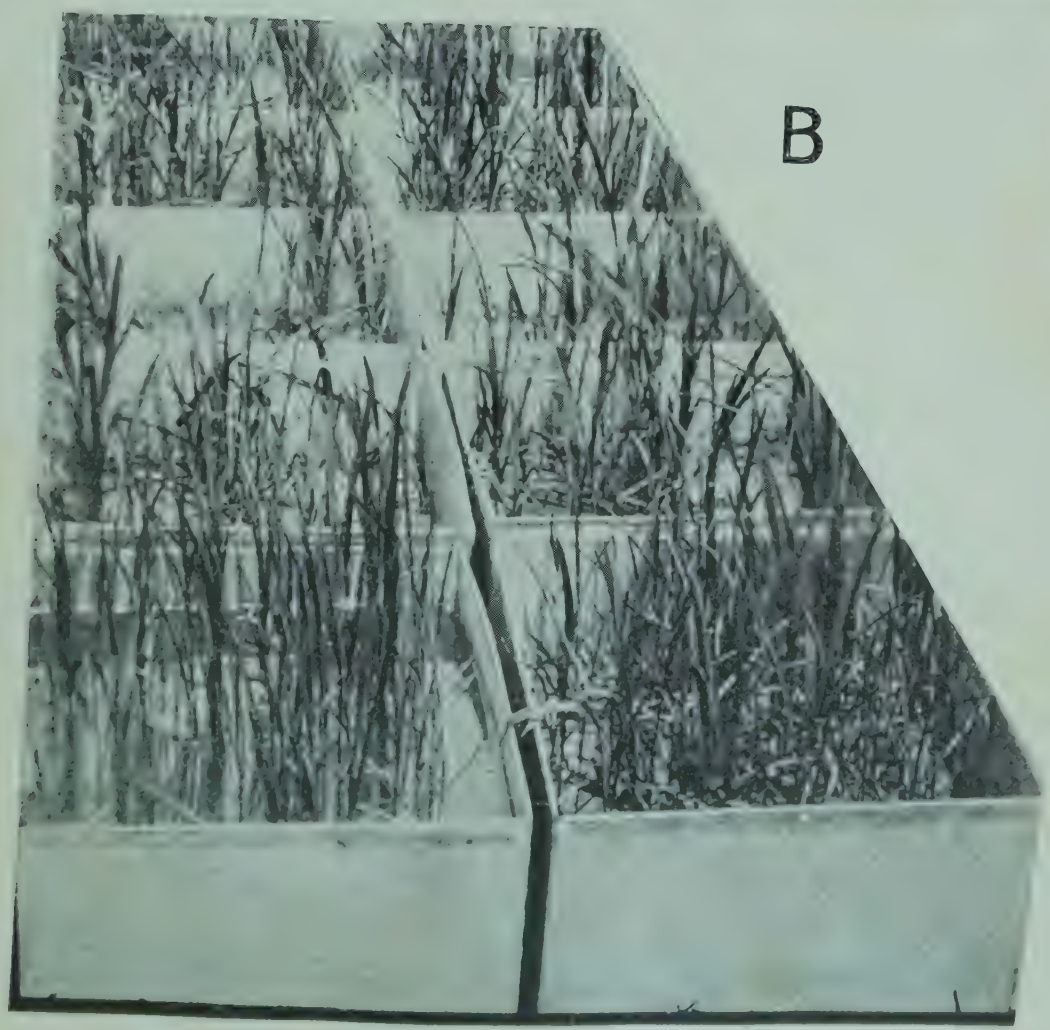
[4] Cattaneo, Achille. op. cit.

PLATE 122

A.—Field showing seedling-blight of rice caused by *Sclerotium rolfsii*. Note dead plants and lack of stand in the two rows in center.

B.—The effect on the stand of rice sown in soil inoculated with *Sclerotium rolfsii* from wheat. The first two cans in the series are uninoculated. Note that seedlings are much more numerous in these two cans.


A


B

A
A
B
B

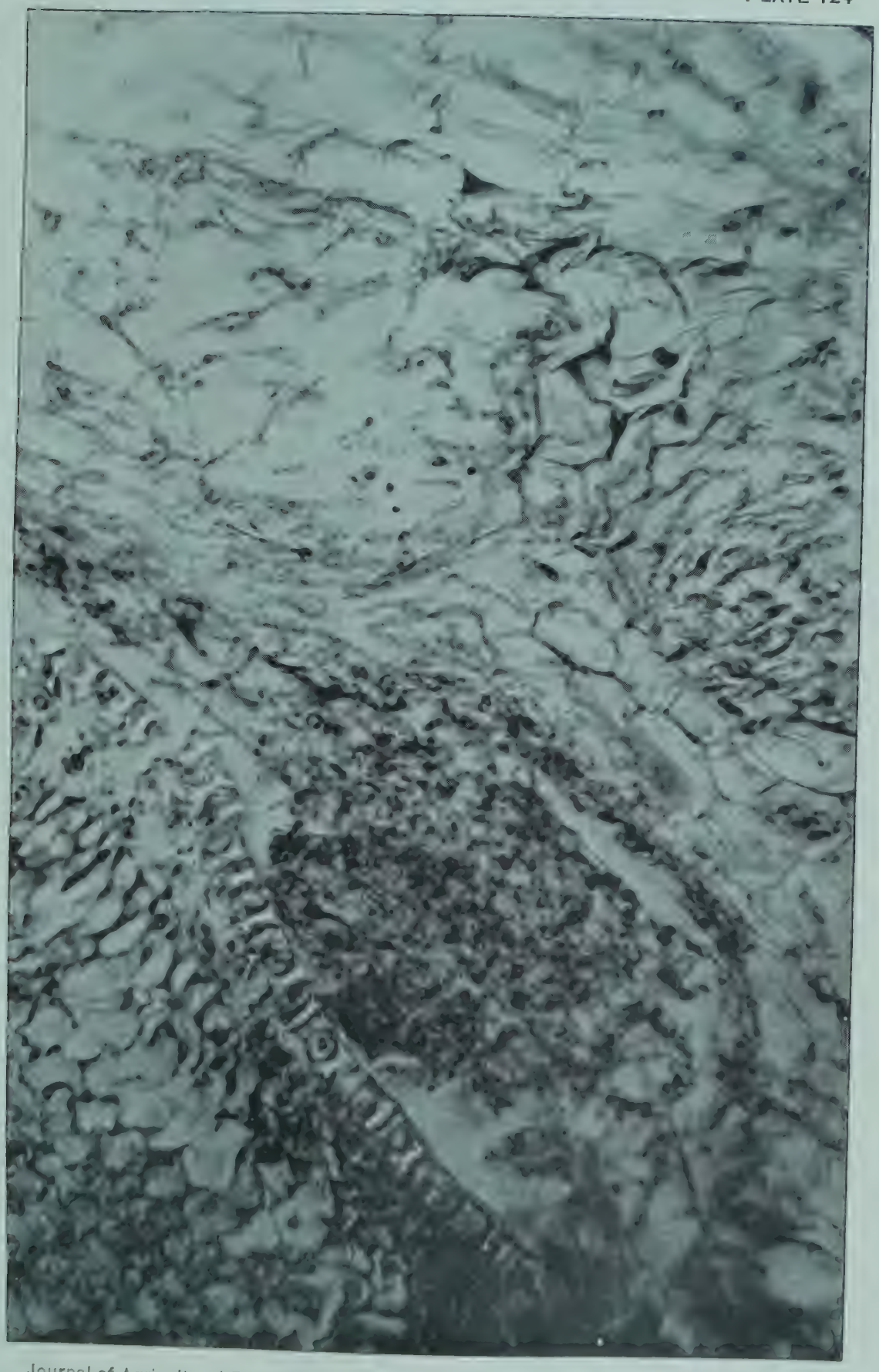
PLATE 123

A.—Rice seedlings in tubes of nutrient agar inoculated with *Sclerotium rolfsii* from rice. The seedlings have been killed.

B.—Healthy rice seedlings on uninoculated nutrient agar.

PLATE 124

Photomicrograph showing the mycelium of *Sclerotium rolfsii* invading the tissues of the basal portion of the stem of the young rice seedling. Note the clump of mycelial fibers in the lower center.



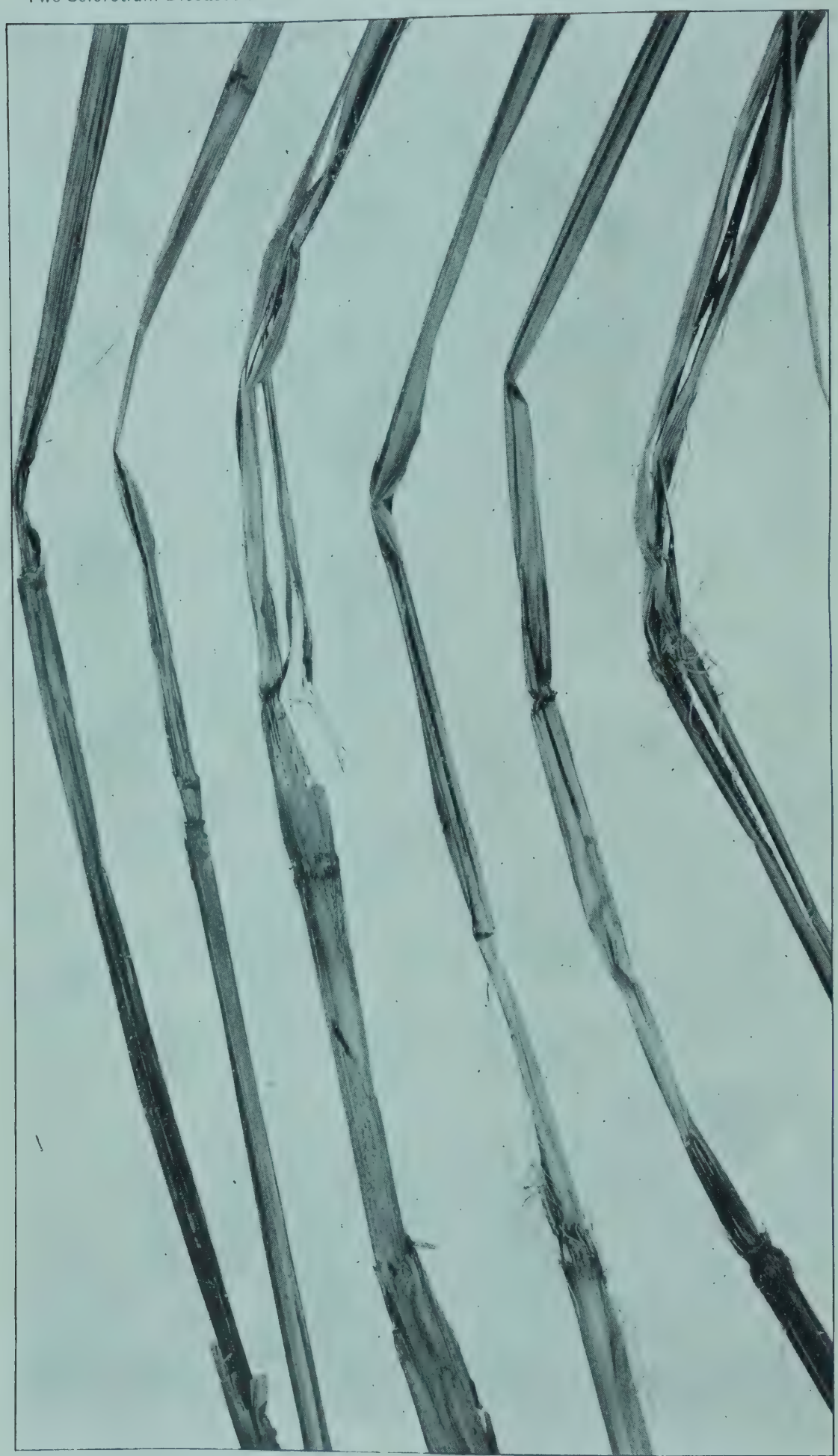
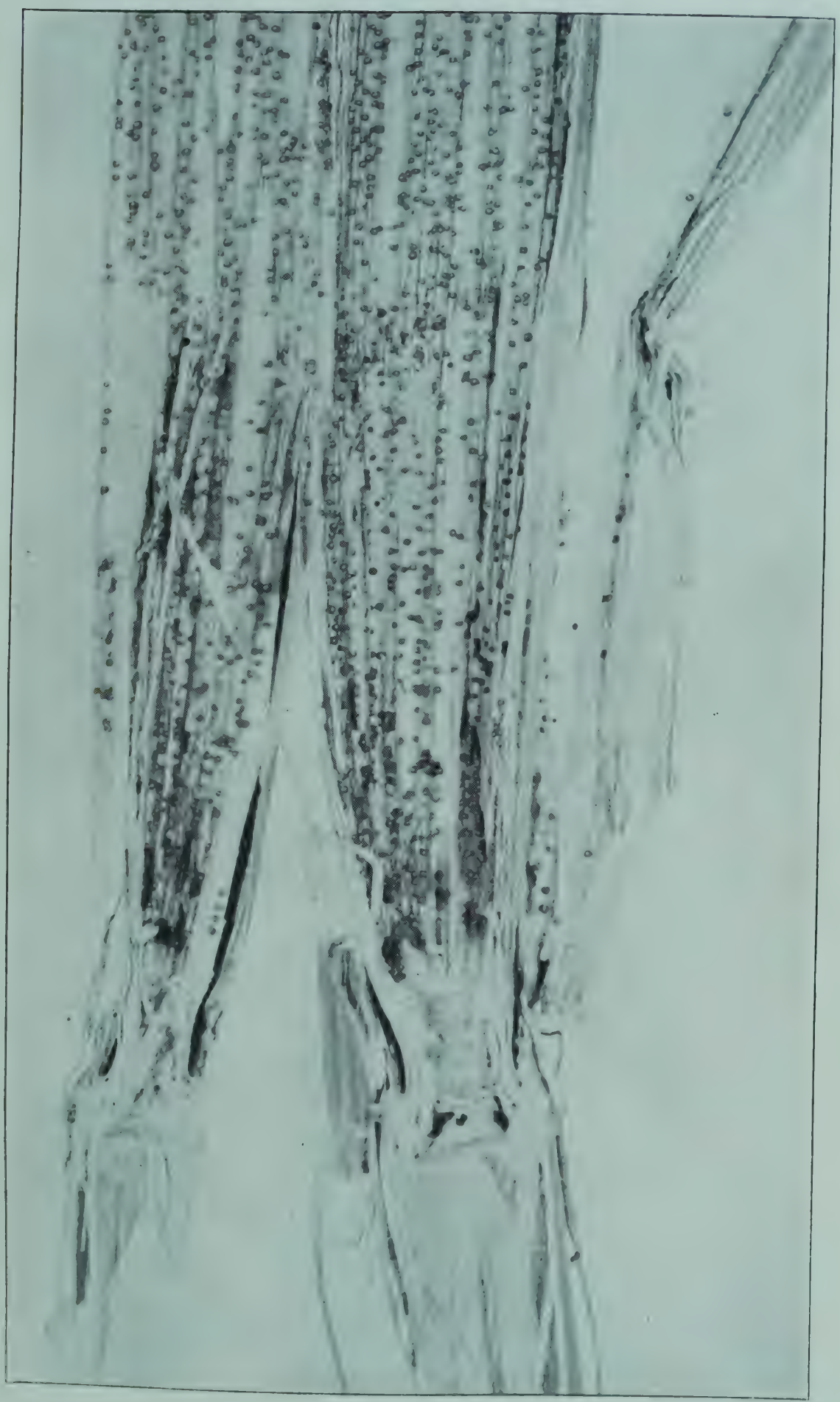


PLATE 125

Stems of Early Prolific rice attacked by *Sclerotium oryzae* Catt. Note that the internodes are completely collapsed.

PLATE 126

Enlarged photograph showing the destroyed inner portion of a rice stem attacked by *Sclerotium oryzae* and the numerous black sclerotia of the organism present.



A BIOLOGICAL STUDY OF THE RED DATE-PALM SCALE, PHOENICOCOCCUS MARLATTI

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INTRODUCTION

The present paper gives the results of a study of the biology of the red date-palm scale made during monthly field inspections in the Coachella Valley, Calif., during the year 1920. This study was conducted with the object of determining the factors in the life history which may be of importance in considering control of this pest. No attempt has been made to determine the details of the life cycle, such as length of instars, variation in length of life cycle, etc., excepting those more important points which bear directly on the general study. Most of the work was conducted at the Government Gardens at Mecca, Calif., as the older trees in these gardens are generally heavily infested with scales, but frequent inspections were made of several of the larger commercial plantings to check up the observations.

DISTRIBUTION

This scale was discovered in 1890 by Dr. C. L. Marlatt at Washington, D. C., on some date palms imported from Algeria, North Africa. In 1899 Mr. T. D. A. Cockerell, while in Washington, studied the material collected by Dr. Marlatt and described the insect as a new genus and species (*Phoenicococcus marlatti* Ckll.). Several years later Mr. Cockerell found small colonies in palms in the gardens at Tempe, Ariz. Since then the infestations have increased, and it has been brought in with practically all of the later introductions of offshoots into California and elsewhere. Distribution of these and other infested offshoots has established the scale in practically every garden of imported palms.

There are over 10,000 imported palms in orchard form in the Coachella Valley now, and practically all are infested.

The quarantine act of 1913[2] affecting interstate movement of infested palms has restricted the distribution of this insect outside of the States

[1] The writer wishes to acknowledge the assistance of Mr. Bruce Drummond and Mr. A. J. Shamblin, of Indio, Calif., who have given freely of their time and practical field experience. In many ways the present work has been corroborated by the observations and experiences of these men.

[2] [AN] ACT TO REGULATE THE IMPORTATION OF NURSERY STOCK AND OTHER PLANTS AND PLANT PRODUCTS; TO ENABLE THE SECRETARY OF AGRICULTURE TO ESTABLISH AND MAINTAIN QUARANTINE DISTRICTS FOR PLANT DISEASES AND INSECT PESTS. ... [Approved August 30, 1912, amended March 4, 1913, and March 4, 1917. *In* U. S. Statutes at Large, v. 37, pt. 1, p. 315–319, 853–854, 1913; v. 39, pt. 1, p. 1165–1166, 1917.

and counties then known as infested, but distribution within this area has been only partially retarded.

There is little probability of the dissemination of the scale by its own movement, as only during its very young stage does it move at all and it could hardly travel from tree to tree at this time.

The most important means of distribution is through the infested offshoots. These offshoots are invariably rather heavily infested and derive the infestation from the bole of the parent tree. Many severely infested offshoots have been found in the propagation houses, where they are placed for rooting.

Of lesser importance is the accidental carriage of the scale by the men at pollination time; and carriage by birds, insects, and the wind, especially in the spring during the so-called migratory period of this scale insect.

ECONOMIC IMPORTANCE

In severe infestations this scale has the habit of massing on the new succulent fruit stems and leaf bases of the palm in such numbers as greatly to weaken their normal development. In fact when the fruit stems are severely attacked the flow of sap is so greatly arrested that the fruit drops just before reaching maturity. Mr. Drummond states that a heavily infested Tazizaoot palm lost its entire crop for three successive seasons at the Mecca gardens. One date-growing company lost approximately 1,200 pounds of fruit from 75 infested palms in 1917. In the early spring during the migratory period the insects mass not only on the fronds but also on the fruit clusters and occasion serious damage. That they also retard the normal growth of the palm is evident.

DESCRIPTION OF STAGES.

In the body of the mature, wine-colored insect may be seen the developing embryos with their six legs, antennæ, dark eye spots, and coiled mouthparts. The young are born alive and issue from the ventral side of the mother scale into a depression in the body wall.

LARVA

The young female larva is flat and oval in shape, pinkish white in color, 0.24 mm. in length, with body segmentation fairly well defined. The antennæ are 6-segmented, the basal segment being very broad and the terminal segment cylindrical. The abdomen is 7-segmented and bears two pairs of setæ on the caudal end, the inner pair being nearly twice as long as the outer. The mouthparts are fully twice the length of the body, exceedingly fine and whiplike.

After remaining under the mother scale for a time the larva crawls out and wanders about in search of a suitable place to feed and then settles for life. The long stylet mouthparts are inserted, and the larva begins

to feed. The body becomes greatly distended with sap and assumes a rounded elliptical shape, rather shiny and yellowish white in color. Fine filaments of "cotton" are given off, first from the lateral glands, and before long the entire body is covered with a cottony mass.

Under this mass the first molt occurs. The cast skin shows a ventral split and the exuvia is thrown off dorsad of the new insect (the mouth-parts remaining attached) and is incorporated in the posterior end of the cottony mass.

The larva now appears somewhat long, oval, light yellow in color, without legs, and with antennæ reduced to small tubercles. Feeding continues and additional "cotton" is secreted, but the insect enlarges so rapidly as to split the cottony mass dorsally and expose the insect. The skin becomes very tight before the second molt.

The second molt is now passed, the cast skin splitting on the ventral side but not always completely thrown off, and the rapidly growing insect (now an immature adult) spreads it so as to leave it incorporated on the upper edges of the old "cotton." The new insect is nearly round but somewhat flattened, 1 to 1.25 mm. in length, wine-red in color, and without apparent appendages or segmentation, but the antennæ under high power are still seen as minute tubercles. The cottony mass formed during the immature period forms a nestlike bed for the scale. Embryos are soon formed within the body and develop rapidly. With the development of the mature scale the wine-red is replaced by a light brown, which starts at the margins; and the color changes to a bronze in the old, dead scale. No embryos have been found in the bronze-colored scale. From field observations it was found that in the cooler part of the year the insect passes from larva to a fully matured adult in approximately a month, and doubtless in the warmer months this period is greatly shortened. As to the life of the scale no definite records have been taken, but field observations would indicate that the scale lives from six to nine months. This is determined by the development of the scale on the fruit stems and leaf bases which become infested in May but have no dead scale on them until late in November.

MALE

Thus far the writer has been unable to discern any distinction as to sex in the larva up to the first molt. From the first molt, however, which in the male form is similar to that described above, there comes a larva similar to but smaller than the female second stage; and from this comes the male pupa, which is rather long, yellowish, and with antennæ and legs folded close to the body. At the third molt the adult male issues, usually through the end of the cottony cocoon. It is shaped not unlike a thrips, with distinct body segmentation, somewhat club-shaped antennæ, rather stout legs, and long, narrow, pointed abdomen, but without wings. The

male, 0.4 mm. long, is very active and moves about much more quickly than the female larva. The number of adult males in proportion to adult females is exceedingly small. Throughout the year, in examining over 300 larvæ and cocoons, the writer found as many as 30 male pupæ but only 8 adult males.

NATURE OF INFESTATION.

The main infestation of scales on a mature palm (Pl. 127) is found on the white living tissues of the leaf bases and fiber bands from 1½ to 3 feet down from the crown, or from the fifth to tenth leaf whorl (Pl. 128). Both inner and outer surfaces are usually infested, and the area may be from ½ inch to 5 inches in width. The scales will be massed together, often in more or less definite order and frequently several layers deep. Infestations of lesser severity will continue on the living tissues of the old leaf bases and fiber bands even to the ground, but as the margin of living tissue narrows the quantity of living scales becomes appreciably less. These insects are usually buried to a depth of 4 or 5 inches under the plant tissues; and as the fiber bands and leaf bases are exceedingly tight, the scale is well protected from heat, dry atmosphere, or control measures. Due to the rapid growth of the palm and definite migratory habit of the scale, the crown of the tree for from three to five leaf whorls, depending upon its size and growth, is kept free of scales. This has been proved by cutting open the crowns of several palms and offshoots. During the greater part of the year (July to April) practically all of the scales are found on the leaf and fiber bases and the fruit stems. The small number found out in the leaf pinnæ are usually of little importance.

That the fruit stems of the current year are found in the area of the main infestation and are tender and succulent at their base explains in part why they are invariably so heavily infested. A number of fruit stems have been removed which were completely massed with scales ⅛ inch thick for a distance of from 6 to 18 inches up from the base. Such infestations seriously injure the development of the fruit. Another important factor is that the fruit stem spathes open and the tender young stem is exposed at the same time that the so-called "spring migratory period" of the scale takes place, thus insuring a complete infestation of that tissue. The buds for the blossoms are laid down deep under the leaf bases in July to September. The bud growth continues through the fall and winter and the spathe pushes out in the spring. Flowering occurs in early April, or late April and early May, depending upon the season and plant variation. Young palms often show greater variation. It is during April, May, and part of June that the greatest migratory period of the scale occurs and the exposed fruit stems become infested. The progress of this infestation was definitely traced during April, May, and

June of 1920. By the latter part of June the fruit stems of the current year were well infested.

Of the infestations on the roots only the so-called superficial roots have been observed by the writer to be infested (Pl. 129, 130). These short, fleshy, gnarled roots, forming above ground, at the base of the bole, are usually under decaying leaf bases and frequently, on old palms, carry a relatively light infestation. They are often so concealed as to be entirely protected. Attention was drawn to an infestation below the ground surface on the bole of a young palm planted in a commercial grove, but on examination it proved to be only a case in which an infested offshoot had been set out and planted deeper than normal, thus burying some of the scale infestation. It has been reported that the scale has been found on the main roots of the palms planted in a very heavy soil at Heber, Calif., which cracked open after each irrigation and left the roots more or less exposed, but no roots have been observed to be infested in the usual sandy soils.

Some scales may be found in the pinnæ of the leaves for a distance of from 2 to 6 feet out from the trunk at all seasons of the year, but the quantity is usually negligible. They undoubtedly get out there during the migratory periods and by the natural pushing out of the growing leaves, but usually they are almost completely controlled by the heat of summer. In some cases scales will persist under the sand deposited in the pinnæ and will reproduce, but in most instances this infestation has been almost negligible after a few hot, dry days. The greatest number are found out on the leaves from the latter part of April until the latter part of June, though they occasionally appear again in September and October during the so-called fall migratory period. The infestations in the leaf pinnæ almost always cause brown pittings on the underside of the pinnæ, and these markings are often used to determine whether a tree is infested or not when it can not be cut into. By July only a few living insects are found in the leaf pinnæ.

Certain varieties of palms show heavier infestations than others. The following generally develop heavy infestations: Horra, Tazizaoot, Rhars, and Rohm Gazal. Palms vary greatly in the tightness of their leaf fiber and bases, but this does not explain the variation in susceptibility to scale attack, as the variety Horra has a relatively tight fiber, but is possibly the most severely attacked.

SEASONAL HISTORY

The insect has been found in all stages of development at all seasons of the year. This would indicate a continual reproduction and overlapping of generations. Though possibly the greater number of active larvæ were observed from March to December, even in January the scale was in all stages of development. In January, however, the mortality among

the young larvæ, either from moisture (mildew) or cold, appeared to be higher than at other times. The greatest development of the scale is possibly reached in the spring (April to June). This is the so-called spring migratory period when the young leave the old areas of infestation and migrate up on the bole and out on the new growth of offshoots to new areas. From the old center of infestation in the area of last year's fruit stems the new center of infestation is moved to the area of the fruit stems of the current year. This is accomplished by actual migration of the young larvæ to the new leaf and fiber tissues above and to the leaf pinnæ. That by June many of these larvæ are killed in the exposed places is evident. From about the middle of April palms with heavy infestations show quantities of cottony masses out on the fronds and new fruit stems and on the new growth of their offshoots. By July this has practically all disappeared.

Mr. Shamblin has often observed a fall migration similar to the spring migration. It is much less severe and the "cotton" of the exposed insects is usually found out on the fronds of the offshoots and parent tree during September and October and disappears almost entirely by the latter part of November. The occurrence of this migration depends entirely upon weather conditions, a cool fall usually bringing on a migration. No fall migration was noted in any gardens during 1919. That this appearance of the insect is usually of relatively small proportion (less than half of the spring generation) and often is completely controlled by weather conditions makes it of much less importance than the spring occurrence.

In Tempe, Ariz., there is much less scale development than in the Coachella Valley, possibly due to a moister climate. This in a way corroborates the observations on the mortality of the young scales during January, 1920. Mildew seems to form quickly in the scale mass when it becomes too moist. The preferred feeding place of the scale (the soft living tissues of the leaf bases and fiber strands) is found to be cool and slightly moist even on the hottest day. This possibly explains the mortality among the exposed scales under the hot, dry condition of summer.

CONDITION OF FRUIT STEMS AT DIFFERENT SEASONS

A study of the progress of the infestation on the leaf bases and fruit stems cut from heavily infested palms at different seasons of the year proved most interesting. It shows clearly how fruit stems of the current year become infested from the spring migration and also shows the development of the scale.

There are usually from 5 to 7 leaf bases between the tip of the crown and the fruit stems of the current year and five leaf bases between the fruit stems of each succeeding year. The leaf bases are arranged in a

spiral on the bole of the palm. The fruit stems come out from behind the leaf bases in a definite area around the bole each year.

August, 1919. Fruit stems of current year (1919) heavily infested with immature and mature (wine-red) stages. Leaf bases above (first two) show rapid decrease (almost clean) in amount of scale of similar development. Leaf bases (third, fourth, fifth) below fruit stem with increasing amounts of old brown and bronze-colored scales, but still some living immature and mature forms.

Fruit stems of 1918 dead and brown but covered at base with old dead scales. Leaf bases below with small margins of scales in all stages of development but decreasing in quantity.

November, 1919. Condition similar to that noted above but with increased amount of scale mass and more reaching brown stage.

April, 1920. (Migratory period beginning.)

Fruit stems of current year (1920) with only few (migratory forms) on outside of bases of spathe.

First leaf base above clean.

First leaf base below clean.

Second leaf base below with only a few very young scales in fiber.

Third leaf base below with young (migrating) forms settling.

Fourth leaf base below with young and a few mature (wine-colored) forms.

Fifth leaf base below heavily infested with wine-colored forms and with a few brown ones.

Fruit stem of 1919 heavily infested in all stages, mostly brown and bronze.

June 2, 1920. (Migratory period about over.)

Fruit stems of current year (1920) lightly infested from 6 to 18 inches from base with immature scales.

First leaf base above clean.

First leaf base below lightly infested with immature forms.

Second leaf base below lightly infested with immature and wine-colored forms.

Third leaf base below with medium infestation; some brown scales but mostly wine-colored and immature forms.

Fourth leaf base below heavily infested with brown and wine-colored forms, some immature at lower margin.

Fifth leaf base below heavily infested, all brown, mostly dead.

Fruit stems of 1919 heavily infested, all brown, dead.

NATURAL ENEMIES

Apparently there are few natural enemies of the red date-palm scale. Of greatest importance is the little beetle identified by Mr. E. A. Schwarz as *Laemophloeus* (*Cryptolestes*) *truncatus* Casey (?) found working throughout the year on the heavier infestations in the Mecca gardens. Occasionally definite galleries of destroyed scales were noted where these beetles were working. This beetle was also found feeding on the scales out on the fruit clusters in a commercial garden during the latter part of April.

In January, 1920, a number of orange-pink dipterous larvæ, possibly those of some species of Itonididae, were found under a leaf base in the midst of a mass of scale insects.

SUMMARY OF BIOLOGY

Following are the important points in the biological study in relation to control:

1. The most important means of distribution is through imported palms and offshoots from infested trees. These offshoots are invariably severely infested and carry the infestation through propagation to new plantings.

2. The main infestation on a mature palm is limited to the area extending 1½ to 3 feet below the crown, the crown tissues being free of scales. Lighter infestations continue on down the bole even to the ground.

3. From July to April practically all of the scales are concealed beneath the leaf and fiber bases and on the fruit stems of the current year.

4. The so-called spring migratory period of the scale is from April to June, and it is at this period that the new tissues and fruit stems become infested from the infested area of last year's fruit stems.

5. The majority of the exposed generations of the migratory periods out on the leaves are killed by the dry, hot weather following the migration, though some may persist in certain protected pinnæ and reproduce a limited number of scales.

6. The scale is found in all stages of development at all seasons of the year on the soft tissues of the leaf base and fiber band tissues. This indicates a continuous overlapping of generations. The duration of each generation is apparently from six to nine months.

CONTROL METHODS

Though the exposed red date-palm scale is readily killed by most contact insecticides its protected position under the leaf bases and tight fiber bands makes control by spray or fumigant most difficult. It is necessary to use a spray which will penetrate deeply between the fiber bands and leaf bases and to repeat the applications consistently in order to reach the maximum number of scales. As shown in the biological studies, it is important to check the migrations of the scale, thus preventing the new tissues and fruit stems from becoming infested.

The formula and spray calendar recommended by Mr. Drummond and Mr. Shamblin are as follows:

Liquor cresolis compositus, U. S. P. ..... 1 part
Distillate or kerosene ..... 4 parts.
Water ..... 50 parts.

If kerosene is used in making the stock solution, only the very best grade should be used, as oils of low specific gravity tend to retard good emulsion, in many cases injuring the offshoot or growing palm.

The spray is best applied by a power sprayer with a good agitator and under 200 to 225 pounds pressure, two leads of 30-foot spray hose being used. Six-foot to 12-foot spray rods with driving-spray nozzles set at

an angle of 45°, should be used. It is advisable to work from an elevated platform in spraying tall palms, rather than to use too long a rod.

Before attempting to spray, the palms should be properly pruned, surplus leaves being removed from the parent plant and the short method of pruning being used on the offshoots.' Where possible, especially in the spring months, as many of the offshoots as are ready should be removed from the parent plant prior to spraying. On old palms the decayed leaf bases and infested superficial roots should be removed. If the dirt has been worked up around the base of the palm during cultivation this should be removed before spraying.

Too much importance can not be given to making a thorough application of the spray. Starting about a foot below the crown of the palm the solution should be so thoroughly applied as to soak all the fiber and completely fill the spaces between the leaf bases from this point to the ground. A slow, careful application made from at least two sides of the trunk is to be preferred to a hasty, incomplete spraying. The spraying of the old leaves to control the infestation in the leaf pinnæ may be limited to severely infested trees and especially immediately following the migratory periods. An average 10-year-old tree with a 6-foot trunk, properly pruned, will take from 15 to 20 gallons of spray.

The proper time to spray is determined and limited by the time the fruit crop is off, the migratory period of the scale, the blooming period, and the time the fruit begins to make sugar. On imported palms (barring seedlings) the fruit is generally all harvested by November 15, the blooming period is from the latter part of March on until May, the spring migration of the scale is from April to June, and the fruit begins to make sugar about August. This permits four sprayings per year, as follows:

First spray period	Jan. 1 to Feb. 15
Second spray period	Feb. 15 to Apr. 1
Third spray period	May 15 to June 30
Fourth spray period	July 1 to Aug. 15

On seedling palms the spray periods will of necessity be governed by the factors mentioned above and will also vary with different varieties. In most cases the spray periods will be of shorter duration, but even then not less than four sprayings should be given.

The treatment of infested offshoots as now practiced consists of thoroughly and completely dipping the cut offshoots in a bath of the foregoing solution. They should be submerged for 15 minutes, taken out and drained for 24 hours, and then submerged a second time for 15 minutes, making 30 minutes altogether in the solution. This solution at a strength of 1 to 50 will not injure the offshoot if left in it 24 hours. Offshoots should also be carefully inspected and sprayed during propagation planting.

PLATE 127

Showing nature of growth of leaves, fruit stems, and offshoots of date palm.

(668)

PLATE 128

Typical infestation of *Phoenicococcus marlatti* at base of leaf stem of date palm.

50936°—21——5

PLATE 129

Superficial roots on old palm generally infested with *Phoenicococcus marlatti*.

PLATE 130

Close-up of superficial roots, showing infestation of *Phoenicococcus marlatti.*

RED DATE-PALM SCALE, PHOENICOCOCCUS MARLATTI: A TECHNICAL DESCRIPTION

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The accumulation of information concerning the identity and distribution of *Phoenicococcus marlatti* Ckll., the biology of which is discussed by Borden (*26*),[2] has been largely due to the efforts of American entomologists and botanists interested either in the insect itself or in the introduction of the date palm into the United States. An examination of the specimens from which this species was described, preserved in the National Collection of Coccidae, shows that it was first collected by Dr.

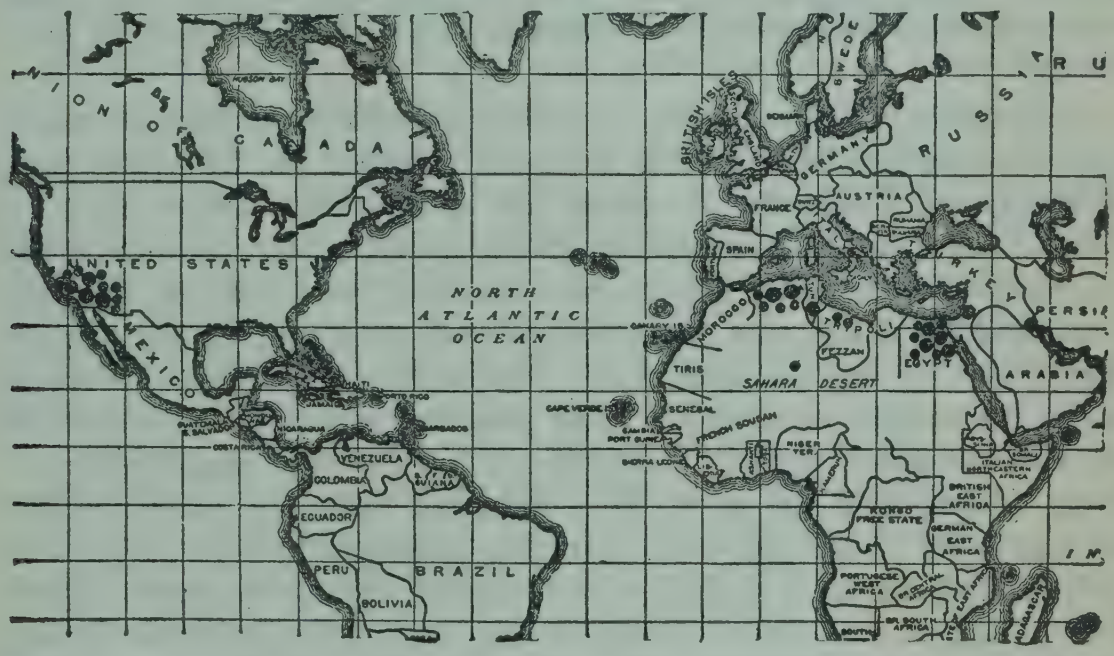

FIG. 1.—Present known distribution of the red date-palm scale (*Phoenicococcus marlatti*), the records based on specimens examined being indicated by large black dots and those based on published records of the occurrence of the species by small black dots.

C. L. Marlatt on August 7, 1890, on young date palms imported probably from Algeria and held at the time in the grounds of the Department of Agriculture in Washington. Subsequently the species has been recorded on many, if not all, of the importations of date palm plants from North Africa and Arabia. The National Collection of Coccidae at present includes specimens from the following definite localities, these being indicated by large round dots on the accompanying distribution map (fig. 1).

ASIA.—Bussorah, Arabian Gulf, specimens collected at Washington, D. C., June 9, 1905; Haifa, Palestine, specimens collected at Washington, D. C., January 30, 1912, by E. R. Sasscer on S. P. I. No. 32721 [3] (*15*).

[1] The drawings illustrating the structural characteristics of the insect described herein have been prepared by Emily Morrison, of the Bureau of Entomology, and the two photographic illustrations by E. R. Sasscer, of the Federal Horticultural Board.

[2] Reference is made by number (italic) to "Literature cited," p. 647–676.

[3] Foreign Seed and Plant Introduction accession number.

AFRICA.—Egypt: General locality, specimens collected at Tucson, Ariz., July, 1904, by R. H. Forbes; general locality North Africa, specimens collected at New York City, July, 1920, by H. B. Shaw on N. Y. F. H. B. No. 595[1]; Cairo, collected at Washington, D. C., February 9, 1912, by E. R. Sasscer; Alexandria, specimens collected at Washington, D. C., May 26, 1920, by H. Y. Gouldman on F. H. B. No. 31843.[2] Tunis: Souf Oases, Sahara Desert, specimens collected June 28, 1907, by W. T. Swingle on S. P. I. No. 21113[3] (*8*). Algeria: General locality, listed as "Algeria or Egypt" by the describer of the species, specimens collected at Washington, D. C., August 7, 1890, by C. L. Marlatt; Orleansville, place of collection not given, specimens collected by T. D. A. Cockerell, in 1899.

NORTH AMERICA.—California: Mecca, specimens collected by A. B. Staubenrauch in February, 1906; the same, specimens collected by A. J. Shamblin, August, 1914; the same, specimens collected at various times during 1918–1920 by A. D. Borden; Imperial Valley, specimens collected by C. J. Brand, April 14, 1908; Indio, specimens collected at Washington, D. C., December 2, 1920, by W. B. Wood on F. H. B. No. 32068.[2]

In addition to these records for which specimens have been examined, the following published records have not been verified by actual study of specimens: Cockerell (*3*) reports the species from Tempe, Ariz., in 1902. Newstead (*4*) describes this species as new under the name *Sphaerococcus draperi* from the Government Gardens, Delta Barrage, Egypt, in 1906, and his description was reprinted with comments in the Agricultural News (*5*) for the same year. Draper (*6*, *p. 12*) lists this species under Newstead's name in the same year and states that it is common throughout lower Egypt. Cockerell (*7*, *p. 191–192*) again reports it from Tempe, Ariz., in 1907. It is discussed from the general locality of Algeria by Trabut (*9*, *p. 68*) in 1910. Lindinger in 1912 (*11*, *p. 247–248*), and again in 1913 (*17*, *p. 689*) reports the species from Algeria, Tripoli, Egypt, and Italy.[4] Essig in 1913 (*19*, *p. 94*) and again in 1915 (*21*, *p. 123–124*) notes its occurrence in Riverside and Imperial Counties in California, as does Cook (*20*) in 1914. The Notice of Quarantine No. 6 of the Federal Horticultural Board of the United States Department of Agriculture (*13*) gives Riverside and Imperial Counties in California, Yuma, Maricopa, and Pinal Counties in Arizona, and Webb County in Texas as localities where the coccid now occurs in the United States. Forbes (*16*) in connection with a discussion of the control of date-palm scales gives Phoenix, Ariz., as a place where this species occurs; and, finally, Pierce (*22*, *p. 162*) in 1917 lists it from Algeria, Egypt, and Sahara as among the dangerous insects likely to be imported into the United States.[5] All of these published localities are indicated on the accompany-

[1] Federal Horticultural Board (Port of New York) accession number, unpublished.

[2] Federal Horticultural Board (Washington, D. C.) accession number, unpublished.

[3] Foreign Seed and Plant Introduction accession number.

[4] Lindinger (*14*) says (translation) "reported once from Italy." It has not been possible thus far to locates this report in any other coccid literature, and Leonardi (*22*) fails to include the species in his list of Italian Coccidae published in 1917.

[5] In an article appearing after the preparation of this description Buxton (*25*) notes the occurrence of this insect in Egypt and Algeria, its importation and establishment in California and Arizona, and his failure to locate it in Mesopotamia where he made an investigation of date-palm insects. He gives a fairly extended discussion of its habits and calls attention to a paper by Trabut (*12*) describing an Algerian disease of date palms caused by this scale insect.

ing distribution map (fig. 1) by small black dots. Besides these, Wilsie (*14*) and Popenoe (*18, p. 151*) discuss this insect without giving definite distribution records. In every case on record, the host of the insect has been some variety of the date palm, *Phoenix dactylifera* L., and all of the evidence now available indicates that it is probably confined to this host in nature.

The preceding definite records of the occurrence of this species indicate a very scattered distribution, but considering its habits and the methods employed by human agencies in distributing its host, it is highly probable that this scale insect actually occurs wherever the date palm is cultivated, as has been suggested by Dr. W. T. Swingle and Prof. S. C. Mason, of the Bureau of Plant Industry, United States Department of Agriculture, both of whom have made collections of date palms for introduction into the United States.

It is not possible at present to give definitely and accurately the relationships of this species to other coccids. It has previously been placed as a member of the subfamily Dactylopiinae, which is obviously made up of a number of only slightly related groups, few of which have shaped themselves with any clearness thus far; and this species will, with little question, be found to be a member of some one of these incompletely defined groups within this subfamily.

The recent study by Ferris (*24*) on the genotype of Sphaerococcus has shown clearly that the species under discussion has no close relationship whatever with Sphaerococcus, that Prof. Cockerell (*1, p. 262; 2, p. 277*) was entirely justified in erecting a new genus for it, and that the conclusions of Newstead (*10, p. 104*) and of Lindinger (*11, p. 247–248; 17, p. 689*) to the effect that this species should be known as *Sphaerococcus marlatti* rather than *Phoenicococcus marlatti* are incorrect.

The technical descriptions of the various stages of both sexes follow.

Phoenicococcus marlatti Ckll.

LARVA (Pl. 131, A–I).—Embryonic larva ready to emerge from female elongate oval, length about 0.27 mm., width about 0.13 mm.; antennæ 6-segmented, placed rather close together at anterior apex of body, front of head between bases straight, basal antennal segment much enlarged, 12 microns long by 25 microns wide, stout truncate-conical, the remaining segments much more slender, approximately cylindrical, the measurements of one antenna in microns as follows: II, 10.5; III, 7; IV, 7; V, 7; VI, 18; legs small, the basal portion stout and the tarsus somewhat swollen, tarsal claw with denticle, the approximate measurements of a middle leg in microns as follows: Coxa, 11; trochanter, 4; femur, 25; tibia, 11; tarsus, 11; claw, 7; digitule about 10.5; beak apparently 1-segmented; with two pairs of thoracic spiracles, these small, each with a relatively long, flat, chitinized bar attached, and each with a single quinquelocular pore just anterior to the opening; derm with seven pairs of long tubular ducts, each terminating in a bilocular 8-shaped pore at its inner end, opening along the body margin, these two pairs on the head, one pair on the mesothorax, one on the metathorax, and one pair each on the first, fourth, and terminal abdominal segments; dorsally with three pairs of similar, but smaller ducts, one on each side about half way

between middle line and body margin, one pair on posterior half of mesothorax, one on anterior half of metathorax, one on first abdominal segment, no types of pores other than these two observed; derm bearing only small, slender setæ, these in longitudinal rows, with a submedian and a submarginal both dorsally and ventrally, and a marginal row, this last having the smallest setæ, with one seta of each row to each segment, at least on the abdomen; a few of the setæ near the antennæ much larger; apex of body terminating with two pairs of very large setæ, relative to those on the body, the outer pair about 53 microns long, the inner about 125 microns; without traces of anal lobes; anal ring small, simple, flattened and extended below, with a more heavily chitinized inner margin, placed apically or a little ventrally, bearing two small setæ above, two near the lower edge of the opening, and two, larger, at the lower edge of the chitinization.

SECOND-STAGE FEMALE (Pl. 132, A–D).—Shape variable, according to stage of development, shortly after first molt somewhat ovate, broadest a little anterior to middle, tapering posteriorly, rounded anteriorly, length at this time 0.32 mm., width about 0.185 mm., later enlarging and approaching more nearly the broad oval form found in the typical adult; derm clearing completely on being treated with caustic potash, showing many small, angularly pointed papillæ, these much less conspicuous in the older swollen specimens, not in distinct close clusters, but scattered along the body margin and in the submarginal area beneath, largest anterior to the antennæ; antennæ placed ventrally near anterior apex of body, nearer to margin than to each other, very much reduced, apparently 1-segmented, the apex slightly invaginated and bearing five small, stout setæ; without traces of the eye spots or legs in mounted specimens; framework of mouthparts large and conspicuous, beak very short and stout, apparently 1-segmented, but this not definitely determinable; spiracles with a circular opening at the outer end of an elongate chitinous bar having a somewhat expanded inner end, and with a loose cluster of 4 to 7 relatively large, quinquelocular disk pores around the opening, the anterior spiracles usually with one or two more of these than the posterior pair; derm along the margin, and in the submarginal region ventrally, with a number of the long tubular ducts already described for the larva, these scattered rather uniformly, varying somewhat in size and much less numerous than the derm papillæ; body dorsally, at the margin, and ventrally with a few longitudinal rows of short and stout, acute setæ; anal ring placed ventrally near the posterior apex of the body, small, simple, the opening somewhat kidney-shaped, retaining a single pair of small setæ, these morphologically at the lower angles of the ring.

ADULT FEMALE (Pl. 133, A, D–M; Pl. 134).—The living specimen, lying on the surface of the host tissue, more or less surrounded by a rim of dense waxy secretion, this ordinarily white, but often discolored through the incorporation of particles of foreign matter; body varying considerably in shape, stout oval, often somewhat broader behind, not strongly convex, the dorsal surface flattened, ventrally excavated along the middle line, presumably to furnish a space for the emergence of the larvæ; body color reddish, except for the central area, this appearing pale greenish yellow; dorsally faintly shining; with some fine, scattered, curved, glassy threads of secretion dorsally, particularly near the margin; color ventrally similar to that of dorsum, with the large groove already mentioned more or less filled with a loose, fluffy secretion of glassy threads similar to those occurring dorsally, but much longer, much more abundant, and curled.

Adult female morphologically stout and broad oval, often nearly circular, length just after molting about 0.5 mm., width about 0.37 mm., maximum length of specimens examined 1.4 mm., width 1.1 mm.; derm just after molting clearing completely on being treated with caustic potash, the derm around the margin showing a broad band of closely crowded, elongate papillæ, each with serrate or denticulate apex, and dorsally, laterally and ventrally longitudinal rows of small, stout setæ; fully matured

adult, as indicated by the measurements given, considerably larger and broader, derm remaining more or less chitinized and yellow brown on being treated with caustic potash, the extent of this apparently depending on the age of the specimens at the time of their separation from the living host; antennæ placed ventrally near the body margin, separated from each other by a distance slightly greater than the width of the framework of the mouthparts, very small, apparently 1-segmented, and of peculiar construction, the apex invaginated for fully half its length, and bearing five short spines at the bottom of the invagination, these protruding little, if any, beyond the mouth of the invagination; legs entirely wanting; framework of mouthparts large, but relatively less conspicuous than in preceding stage; beak small and stout, conical, segmentation not definitely determinable; spiracles with an elongate bar, expanded at inner end, with a loose cluster of from 12 to 15 quinquelocular disk pores around the opening, and with a varying number of similar pores scattered beyond each spiracle towards the margin, these particularly noticeable opposite the anterior pair; derm with the papillæ remaining closely crowded at posterior and anterior apices of body, but much more scattered along lateral margins where the principal expansion due to growth evidently occurs; derm with only one other type of pore in addition to the spiracular pores, this a long tubular duct with bilocular inner end, similar to those of larva and second stage, but much more abundant, varying somewhat in size, a few scattered dorsally, but mostly occurring along the margin and ventrally, never in clusters, although more numerous in certain areas, as throughout the ventral groove region, than in others; derm with an occasional small seta, these most numerous in the midventral abdominal area; anal ring placed ventrally near the posterior apex of the body, small, simple, heavily chitinized, retaining the two lower setæ only as in preceding stage.

MALE LARVA.—Apparently indistinguishable from that of female.

MALE SECOND-STAGE LARVA (Pl. 131, J–M).—Rather elongate ovoid, broadest before the middle, length about 0.32 mm., width about 0.18 mm., probably flattened; antennæ placed near anterior apex of body, separated by the width of the framework of the mouthparts, small and short tubular, apparently 1-segmented, more or less invaginated at apex; legs entirely wanting; framework of mouthparts very large and conspicuous, beak small, short conical, segmentation not determinable; spiracles small, with a heavy chitinous bar somewhat expanded at inner end, each accompanied by a single, normally quinquelocular disk pore; derm with some of the tubular ducts of other stages along the margin of the body and in the submarginal area ventrally; derm, at least on the abdomen, with eight longitudinal rows of small setæ, one seta of each row to a segment, two rows dorsal, two marginal, and four ventral; derm with a few small papillæ around the posterior apex of the abdomen, these quite inconspicuous; anal ring small, simple, kidney-shaped, retaining the lower two small setæ, and showing a slight chitinized connection with the still lower pair of large setæ, but these well removed from the ring proper.

MALE PUPA (Pl. 131, N; 132, E).—Elongate oval, length about 0.36 mm., width about 0.16 to 0.18 mm.; antennæ and legs large and conspicuous, showing distinct segmentation in stained specimens, all these long conical, tapering from base to apex, the antennæ placed at the anterior apex of the body and protruding laterad and a little backwards, showing five distinct segments; anterior legs pointing cephalad, parallel and somewhat overlapping the antennæ, intermediate legs directed backwards and outwards, posterior legs extending backwards, nearly parallel and nearly reaching the apex of the abdomen, all the legs showing five distinct segments in stained specimens; mouthparts wanting, the derm with a large, roughly circular, chitinized area at the point where these occur in other stages; spiracles small and stout, without quinquelocular or other pores; derm, so far as can be noted, entirely without specialized secreting pores or ducts; with eight rows of tiny circles, these

possibly normally spine-bearing, two dorsally, two ventrally, two close to margin dorsally, and two submarginal ventrally; body terminating in a heavy, short, cylindrical protuberance having an irregular transverse opening in its center.

ADULT MALE (Pl. 133, B, C, N-R).—Elongate, slender, sides nearly parallel, rounded anteriorly and tapering to a point posteriorly, dimensions from specimens mounted alive in balsam, length 0.42 mm., width 0.14 mm.; antennæ stout, placed close together at anterior apex of body, the two bases separated by perhaps half the width of the basal segment, this much broader than long, the second segment stout, somewhat larger apically, the third segment with a slender stalklike base, this with several grooves and ridges running around it, but the apical three-fifths strongly expanded, fourth segment also triangular, but stouter at base than third, remainder of antenna, in specimens mounted in balsam while alive, appearing as three distinct but closely united segments, this appearance much less conspicuous in specimens treated with caustic potash and stained, but still indicated, the antennæ, therefore, probably 7-segmented with the five terminal ones forming an elongate club terminating in a stout button appearing more or less distinctly set off by a joint between it and the club proper, as if it might be still another segment; so far as can be determined, with two long, slender setæ on II, III, and IV, with four similar at the apex of the club, and with one stout spine on V, one, longer, on VI, and two, still larger, on VII; measurements of one antenna in microns as follows: I, 14.2; II, 17.5; III, 14; IV, 10.7; V, 10.4; VI, 7.4; VII, 14.3 (total of club apex 32.1); legs short and stout, tibia and tarsus of each bearing one or more large, round, apparently sensory pores; lengths in microns of outer portion of a middle leg: Femur, 32; tibia, 18; tarsus, 14.3; claw, 10.7; digitules about 14; claw with denticle, digitules slender, slightly knobbed at tips; eyes simple, only one pair present, these as spots on margin a little posterior to the antennæ; mouthparts entirely wanting; wings entirely wanting; spiracles small, the bar stout, without any accessory pores; derm, so far as can be determined, entirely without specialized secreting pores, bearing, however, a number of tiny chitinized circles, these occurring singly, but probably in definite arrangement, normally seta-bearing; penis elongate, slender, apparently cylindrical, the surface roughened; sheath elongate, tapering, pointed, length about 30 microns, width close to base about 7.5 microns, suddenly expanding at base to about 14 microns.

In preparation of the preceding description specimens of one or more stages of the species from each of the lots of material now in the National Collection of Coccidae have been examined.

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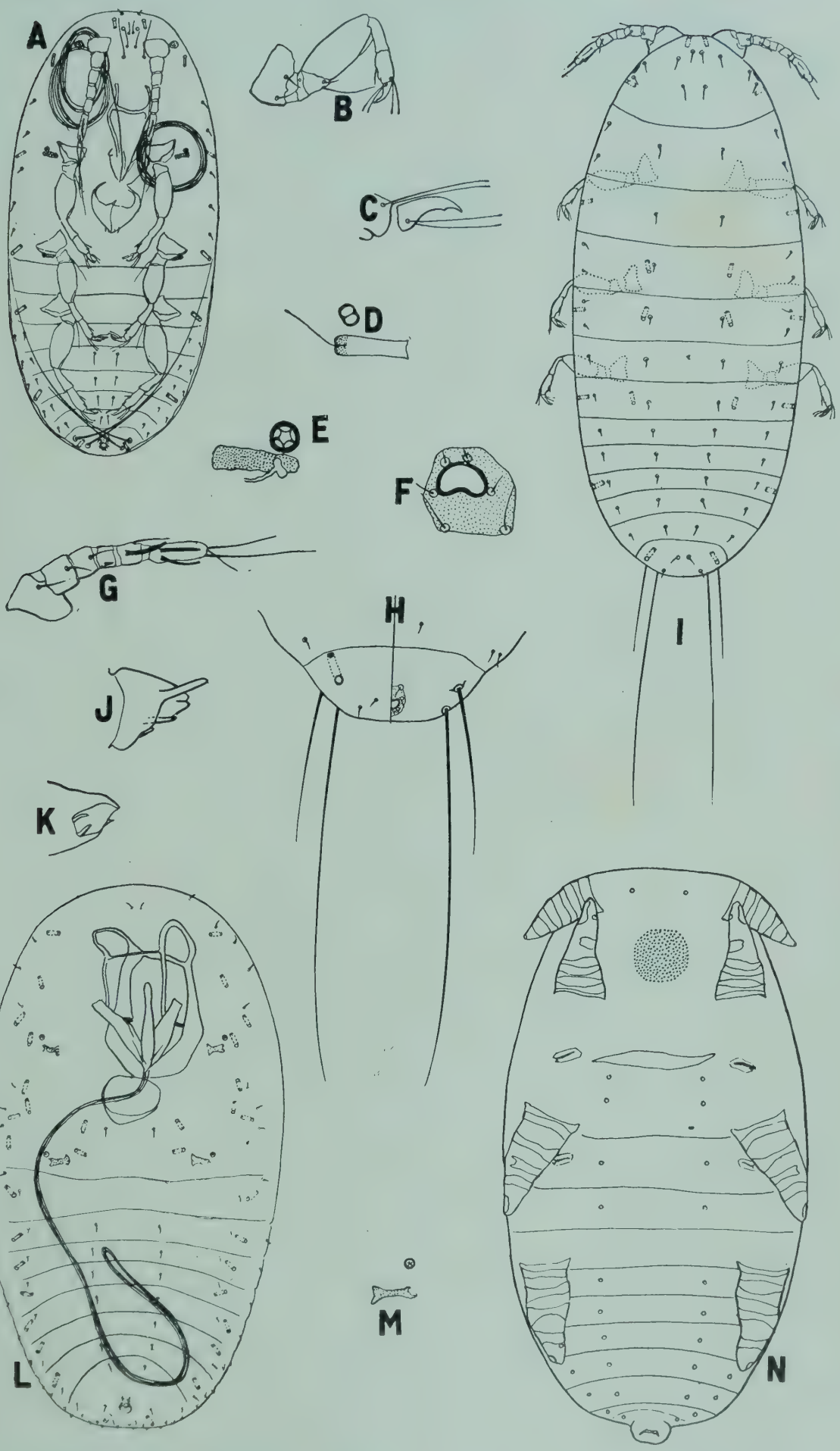
PLATE 131

Phoenicococcus marlatti:

A.—Embryonic larva. × 220.
B.—Larval leg. × 450.
C.—Larval claw. × 1,350.
D.—Larval tubular duct. × 1,350.
E.—Larval spiracle. × 1,350.
F.—Larval anal ring. × 1,350.
G.—Larval antenna. × 450.
H.—Larva, apex of abdomen. × 450.
I. Larva, outline from above. × 220.
J.—Male second-stage larva, antenna. × 1,350.
K.—Same, another antenna. × 1,350.
L.—Male second-stage larva, outline of body from beneath. × 220.
M.—Male second-stage larva, spiracle. × 450.
N.—Male pupal skin. × 220.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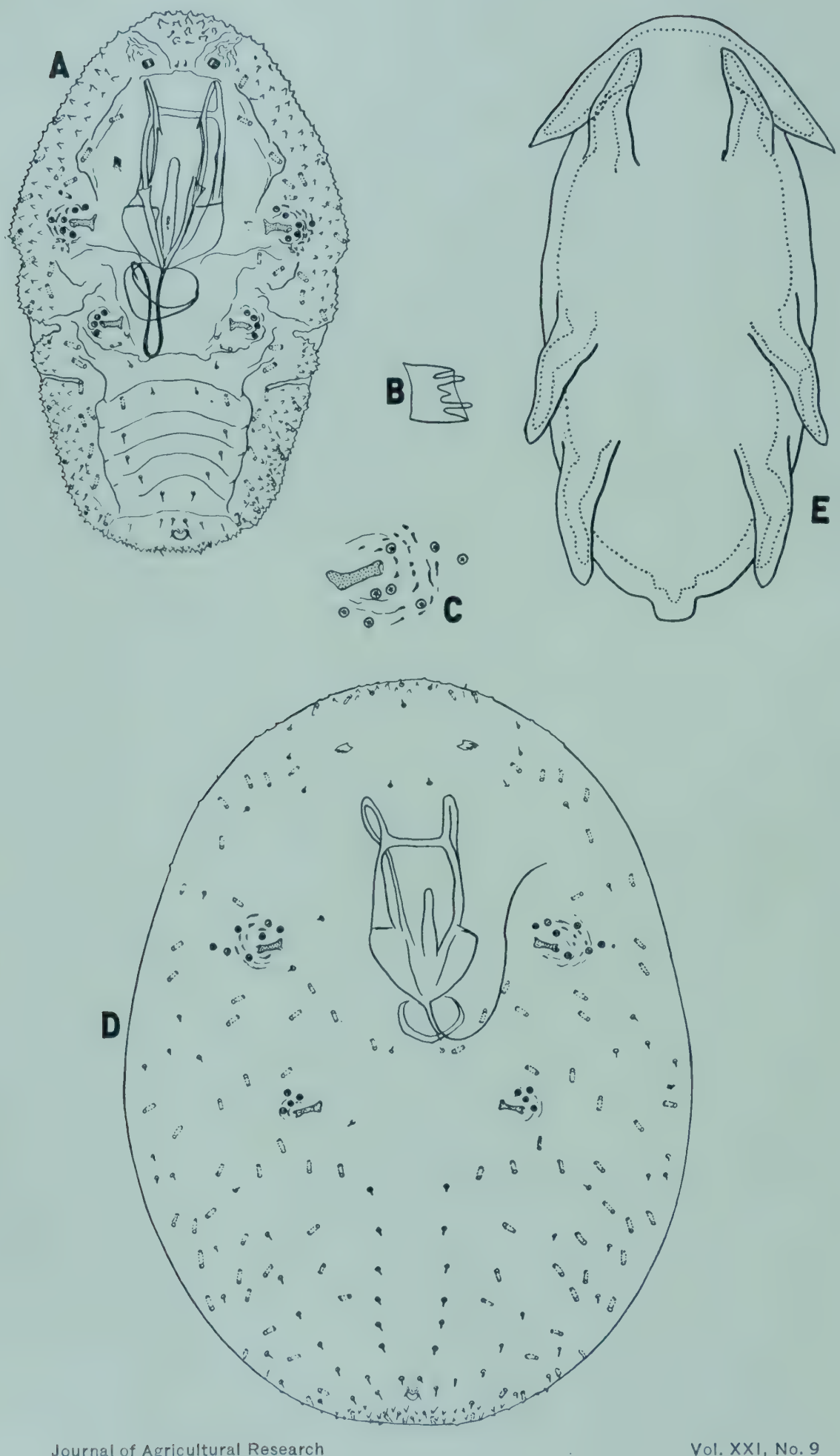
A
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PLATE 132

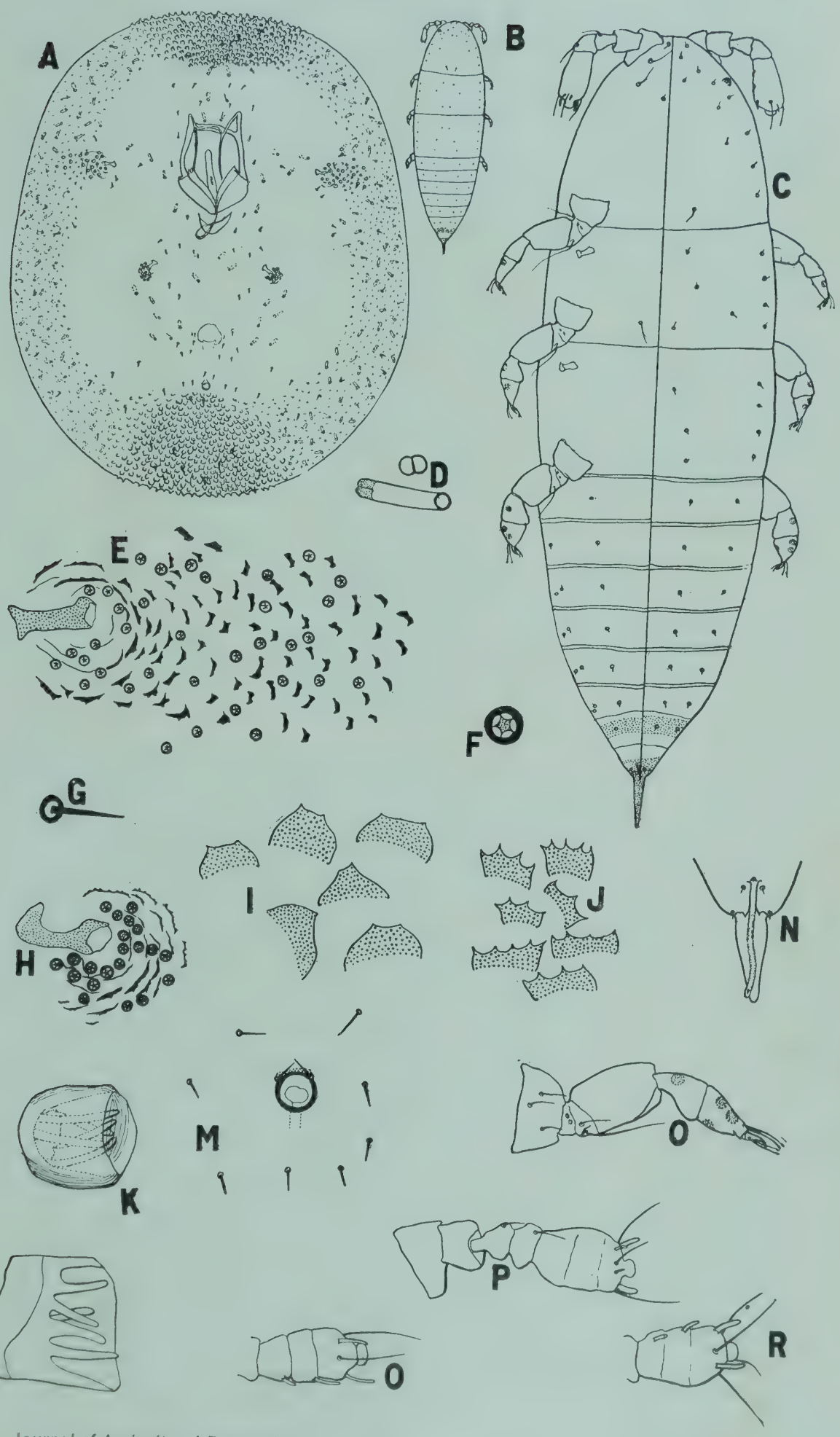
Phoenicococcus marlatti:

A.—Female, second-stage larva, just after molt. × 220.
B.—Same, antenna, optical section. × 450.
C.—Same, anterior spiracle. × 450.
D.—Same, outline of body after expansion to normal size. × 220.
E.—Male pupa, outline, showing half-formed adult male inclosed. × 220.

PLATE 133

Phoenicococcus marlatti:

A.—Adult female, outline from beneath. × 70.
B.—Adult male, outline from above for comparison with female. × 70.
C.—Adult male, outline from above. × 220.
D.—Adult female, tubular duct. × 1,350.
E.—Adult female, anterior spiracle. × 450.
F.—Adult female, spiracular pore. × 1,350.
G.—Adult female, derm seta. × 1,350.
H.—Adult female, posterior spiracle. × 450.
I.—Adult female, derm papillæ from old, distended body. × 1,350.
J.—Adult female, papillæ from body before distension. × 1,350.
K.—Adult female, antenna. × 1,350.
L.—Same, crushed and flattened, showing the setæ. × 1,350.
M.—Adult female, anal ring and surrounding area. × 450.
N.—Adult male, penis and sheath. × 450.
O.—Adult male, leg. × 450.
P.—Adult male, antenna from cleared and stained specimen. × 450.
Q.—Tip of same from specimen mounted alive, showing apparently distinct segmentation. × 450.
R.—Same, tip of antenna figured at P, showing opposite surface. × 450.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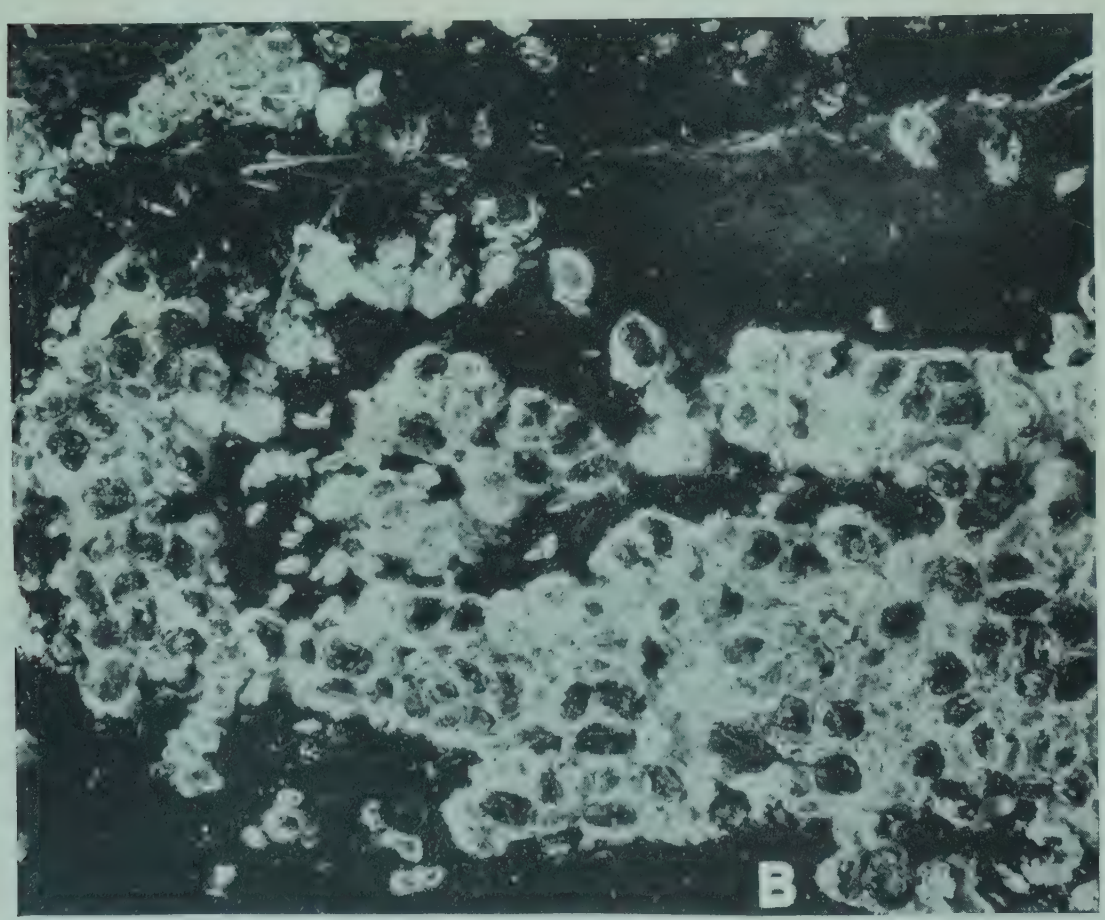
B

PLATE 134

Phoenicococcus marlatti:

A.—Mass of females inside base of palm leaf.
B.—Portion of the same mass still more enlarged.

50936°—21——6

SOME OBSERVATIONS REGARDING EOSINOPHILES

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During the past few years our knowledge of the relationship of a parasitic infestation and an eosinophilia has been undergoing considerable revision. Not so very long ago the presence of an unusually large number of eosinophiles was considered almost diagnostic of a severe infestation with some form of external or internal parasites. Also an absence of an eosinophilia presented very strong evidence that the animal was almost if not entirely free from parasitism.

The observations reported here are based on a study of 40 cases. From 1 to more than 20 blood examinations have been made on the individual cases, some of which extended over a period of about 18 months. In all the cases the animals were carefully autopsied and a close search for internal parasites was made. None of the animals were harboring external parasites.

The usual precautions for drawing the blood and making the examinations were observed. The place chosen for procuring the blood was the under surface of the tail. In case this failed, the skin was shaved at the shoulder and the incision made there. As a rule, Tallquist's hemoglobin scale was used, although Gower's method was used in some instances. Both red and white cells were counted in the same preparation, a double-ruled Neubauer counting chamber being used in most cases. For the differential count Giemsa's stain was ordinarily used, but Wright's and Jenner's were sometimes used. Five hundred cells were usually counted, although sometimes 1,000 were counted.

The writer is indebted to Dr. Edward Records for checking some of the differential counts. While the two counts did not absolutely check, they were very close. It is not to be expected that even two counts by the same person on the same slide would be identical.

[1] Resigned June 1, 1920.

TABLE I.—*Blood counts of horses in apparent good health*

Tag No.	Date.	Hemoglobin.	Erythrocytes.	Leucocytes.	Lymphocytes.	Polymorphonuclears.	Mononuclears.	Eosinophiles.	Mast cells.	Transitionals.
	Apr. 30, 1918	70	6,572,000	7,130	38.4	51.0	4.0	5.4	1.2	0
	May 6, 1918	77	7,348,000	6,920	40.8	44.6	8.4	5.6	.6	0
	May 18, 1918	90	7,420,000	6,300	41.6	54.2	4.0	.2	0	0
	May 28, 1918	95	8,000,000	9,360	48.2	46.6	3.4	1.6	.2	0
	June 4, 1918	97	8,800,000	8,000	34.6	50.2	10.2	3.0	2.0	0
	June 14, 1918	101	7,960,000	7,260	34.0	51.4	10.0	3.4	1.2	0
	June 19, 1918	103	9,126,000	9,760	30.0	63.8	4.4	1.4	.4	0
673	June 25, 1918	97	7,704,000	5,940	29.4	53.6	6.8	9.2	1.2	0
	July 9, 1918	105	9,010,000	6,660	33.2	51.0	7.2	8.2	.4	0
	July 23, 1918	80	7,642,000	5,850	26.4	56.2	8.0	9.4	0	0
	Aug. 6, 1918	100	7,888,000	7,740	24.4	63.4	9.2	3.0	0	0
	Aug. 26, 1918	94	6,432,000	9,777	33.2	54.4	8.0	4.2	.2	0
	Oct. 8, 1918	100	7,880,000	8,349	21.4	65.2	11.4	2.0	0	0
	Oct. 27, 1918	100	6,888,000	9,861	38.8	39.6	14.6	7.0	0	0
	Dec. 22, 1918	75	7,296,000	7,633	37.4	55.2	6.2	1.2	0	0
634	Oct. 8, 1918	92	7,880,000	9,873	22.6	68.2	9.2	0	0	0
15	May 29, 1919	100	7,964,000	8,291	33.2	60.2	4.2	2.4	0	0
	Dec. 16, 1918	90	7,842,000	7,383	37.6	54.2	5.6	2.6	0	0
	Jan. 3, 1919	100	8,306,000	9,471	30.2	60.0	5.8	4.0	0	0
	Mar. 1, 1919	90	7,684,000	9,021	31.8	57.6	10.2	2.4	0	0
2	Apr. 8, 1919	90	7,964,000	9,769	41.2	52.4	4.4	2.0	0	0
	Apr. 25, 1919	90	7,964,000	7,783	28.2	61.4	7.4	3.0	0	0
	May 16, 1919	100	8,264,000	5,396	30.0	63.2	4.2	2.6	0	0
	May 27, 1919	100	8,032,000	5,387	31.3	57.4	6.6	4.2	0	0
	Sept. 3, 1918	98	6,912,000	8,888	40.2	54.2	3.0	2.6	0	0
	Oct. 10, 1918	90	6,834,000	9,873	37.2	53.0	13.0	6.8	0	0
754	Nov. 2, 1918	88	7,432,000	5,961	34.6	55.4	8.4	1.6	0	0
	Dec. 22, 1918	100	8,264,000	9,666	46.2	49.0	3.6	1.0	.2	0
702	Oct. 27, 1918	85	7,480,000	8,677	63.2	27.4	8.2	1.2	0	0
	May 20, 1918	95	8,288,000	9,400	29.8	63.2	4.4	2.0	.6	0
	May 28, 1918	100	7,216,000	7,920	37.6	53.2	2.6	6.4	.2	0
	June 3, 1918	106	9,260,000	8,330	36.6	50.4	6.2	5.8	1.0	0
	June 12, 1918	105	10,624,000	8,280	29.2	60.2	6.0	3.6	1.0	0
	June 17, 1918	104	10,496,000	8,000	31.2	50.2	12.2	6.2	.2	0
501	June 25, 1918	89	6,688,000	5,580	31.2	54.4	10.2	3.8	.4	0
	July 9, 1918	93	8,340,000	9,360	42.8	50.4	2.2	4.4	.2	0
	July 6, 1918	100	6,384,000	7,200	27.3	56.2	10.2	6.3	0	0
	July 26, 1918	100	7,634,000	10,326	35.4	42.2	12.0	10.4	0	0
	Oct. 27, 1918	90	6,408,000	6,339	36.2	47.8	13.2	2.8	0	0
	Dec. 16, 1918	80	6,848,000	9,333	38.8	46.2	6.2	8.6	.2	0
	Dec. 22, 1918	90	7,362,000	8,431	40.0	37.0	7.8	15.2	0	0
	Jan. 2, 1919	80	7,642,000	4,420	34.2	38.2	7.4	20.2	0	0
	Jan. 28, 1919	100	6,973,000	8,821	24.6	53.4	8.0	14.0	0	0
8	Mar. 1, 1919	90	7,552,000	8,361	23.4	57.8	3.2	15.6	0	0
	Mar. 25, 1919	90	7,244,000	5,391	37.2	40.4	6.4	16.0	0	0
	Apr. 19, 1919	90	7,648,000	7,291	26.3	52.6	3.2	17.4	0	0
	Apr. 25, 1919	80	7,288,000	11,291	26.4	52.8	3.6	17.2	0	0
	May 26, 1919	90	7,648,000	9,361	23.2	56.6	3.8	16.4	0	0
	June 2, 1919	90	7,932,000	8,341	32.2	61.4	5.0	1.4	0	0
	Apr. 8, 1919	90	7,860,000	4,381	37.8	54.2	3.6	4.4	0	0
9	July 5, 1919	100	7,694,000	5,363	30.2	62.4	6.0	1.4	0	0
	Mar 18, 1920	100	8,064,000	7,010	No count made.					
	Sept. 18, 1917	105.6	11,972,000	5,330	46.2	36.2	10.2	7.0	.4	0
	Sept. 25, 1917	95	8,584,000	10,666	47.8	37.0	7.4	7.6	.2	0
	Sept. 28, 1917	95	8,032,000	7,333	38.0	50.4	6.4	4.6	.2	0
570	Oct. 6, 1917	60	5,122,400	1,780	29.8	67.6	2.2	.4	0	0
	Oct. 11, 1917	70	5,824,000	7,555	57.2	26.6	13.2	3.0	0	0
	Oct. 24, 1917	.....	5,072,200	4,666	46.8	46.8	6.0	.4	0	0

TABLE I.—*Blood counts of horses in apparent good health*—Continued

Tag No.	Date.	Homoglobin.	Erythrocytes.	Leucocytes.	Lymphocytes.	Polymorphonuclears.	Mononuclears.	Eosinophiles.	Mast cells.	Transitionals.
	Nov. 27, 1917	65	6,228,000	5,620	45.8	47.4	6.6	.2	0	0
	Apr. 30, 1918	90	6,912,000	7,480	44.3	37.8	10.1	7.1	.7	0
	May 6, 1918	90	8,080,000	9,430	50.4	39.0	7.6	3.0	0	0
	May 10, 1918	80	10,420,000	9,760	36.0	51.0	6.8	4.6	1.2	0
	May 16, 1918	80	8,040,000	9,460	37.2	48.2	5.2	6.6	.8	0
	May 24, 1918	80	7,784,000	8,000	28.0	50.2	10.0	11.2	.6	0
	June 3, 1918	100	8,840,000	7,420	26.4	64.2	5.8	3.6	0	0
570	June 12, 1918	100	7,482,000	7,020	38.4	51.8	7.0	2.6	.2	0
	June 18, 1918	100	8,342,000	6,430	33.4	58.2	5.6	2.4	.4	0
	June 24, 1918	101	9,280,000	4,500	28.0	61.6	6.8	3.6	0	0
	July 8, 1918	95	8,684,000	9,360	36.4	58.2	4.4	1.0	0	0
	July 23, 1918	100	8,560,000	5,588	23.2	72.4	2.4	2.0	0	0
	Sept. 3, 1918	90	7,704,000	8,000	33.4	60.2	4.2	2.0	.2	0
	Oct. 10, 1918	100	8,324,000	5,386	38.6	48.4	10.0	2.6	.4	0
	Oct. 27, 1918	100	7,264,000	11,133	32.0	59.2	5.6	3.2	0	0
7	Dec. 16, 1918	80	7,208,000	6,339	41.4	48.6	7.2	2.4	.4	0
	Feb. 22, 1919	90	6,974,000	8,360	32.6	57.0	8.2	2.2	0	0
13	Mar. 8, 1919	90	7,382,000	5,981	33.4	61.2	2.8	2.6	0	0
	Mar. 25, 1919	80	7,164,000	5,089	22.0	67.2	7.2	3.6	0	0
633	May 2, 1918	70	8,490,000	11,400	33.6	54.6	4.4	6.4	1.0	0
1	Nov. 14, 1918	90	7,232,000	8,169	26.6	55.2	5.2	13.0	0	0
	Average..	90.4	7,783,300	7,693	34.8	52.9	6.8	5.3	.2	0

No. 673.—Aged, healthy gelding in good physical condition.

At autopsy, December 30, 1918, there were found 1 *Gastrophilus* spp., 3 Setaria in the abdominal cavity and a few Strongylus and Cylicostomes in the cecum and colon.

No. 634.—Normal gelding 12 years old.

At autopsy, October 26, 1918, not a parasite of any kind was found.

No. 15.—Normal 15-year-old mare.

At autopsy, May 29, 1919, about 30 *Gastrophilus* spp. were in the pylorus. There was a moderate infestation with Strongylus and Cylicostomes in the cecum and colon. Two *Setaria equina* were found free in the abdominal cavity.

No. 2.—Normal aged gelding.

At autopsy, May 27, 1919, about 350 *Gastrophilus* spp. were found in the pylorus; 1 *Ascaris equorum* was in the duodenum; there was a moderate infestation of Strongylus and Cylicostomes in the cecum and colon; and 25 Setaria were found in the abdominal cavity. There was a severe aneurism of the anterior mesenteric artery which contained live embryos.

No. 754.—Normal 15-year-old mare.

At autopsy, December 30, 1918, there were found 50 *Gastrophilus* spp. in the pylorus and moderately severe infestation with Strongylus and Cylicostomes in the cecum and colon.

No. 702.—Healthy 10-year-old gelding.

At autopsy, December 19, 1918, there were found 10 *Gastrophilus* spp. in the pylorus, about 75 Strongylus in the cecum and colon, and 1 Strongylus and 4 *Setaria equina* in the abdominal cavity.

No. 501.—Aged, healthy mare.

At autopsy, December 16, 1918, there was found a moderate infestation of Strongylus and Cylicostomes in the cecum and colon.

No. 8.—Aged, healthy gelding.

At autopsy on May 26, 1918, there were found about 150 *Gastrophilus* spp. in the stomach and pylorus, a moderate infestation of Strongylus and Cylicostomes, and a small aneurism of the anterior mesenteric artery containing live embryos.

No. 9.—Healthy 12-year-old mare.

At autopsy on March 18, 1920, there were found about 150 *Gastrophilus* spp., a severe infestation with Strongylus and Cylicostomes, and a small worm-free aneurism of the anterior mesenteric artery.

No. 570.—Five-year-old gelding in excellent condition.

At autopsy, December 16, 1918, there were found 6 *Gastrophilus* spp. and a most severe infestation of Strongylus and Cylicostomes. The animal showed no symptoms of the infestation. About two months before autopsy he was corralled with wormy colts, and it seems likely that the infestation came at that time from them.

No. 7.—Aged, healthy mare.

At autopsy, December 20, 1918, there were found a few *Gastrophilus* spp., 2 Setaria in the abdominal cavity, small number of Strongylus and Cylicostomes in the cecum and colon, and a few Oxyuris in the rectum.

No. 13.—Aged, healthy animal.

At autopsy, April 5, 1919, there were found 150 *Gastrophilus* spp., 1 Setaria, a few Strongylus and Cylicostomes in the cecum and colon, and a very large aneurism in the anterior mesenteric artery containing many live embryos.

No. 633.—Young, healthy gelding.

At autopsy, July 8, 1918, there were found a few *Gastrophilus* spp. and a moderate infestation of the cecum and colon with Strongylus and Cylicostomes.

No. 1.—Aged, healthy mare.

At autopsy, December 12, 1919, there was found a severe infestation of the cecum and colon with Strongylus and Cylicostomes.

DISCUSSION

The percentage of eosinophiles in the peripheral blood of healthy horses varies greatly even when the examinations are made at frequent intervals. In studying Table I, one can readily see that if certain individual examinations were picked out, exceedingly varying results might be obtained. This fact possibly accounts for many of the different

conclusions arrived at by many writers regarding the percentages of eosinophiles in the blood of normal horses or horses suffering from various diseases. Another fact that is apparently often if not always overlooked is the resistance of the animal. Weinberg and Seguin (*10*) [1] have pointed out that the eosinophiles possess specific attraction against certain toxic substances, especially those of parasitic origin. It would seem from this that if the resisting power of the animal were high the eosinophiles would be plentiful in parasitic diseases. If the resistance were low the eosinophiles would be few.

In general it can be said that in the various species of animals the physiological functions of the leucocytes are essentially the same. If this be so, cattle must be very severely infested with parasites or else their resistance is high. Dimmock and Thompson (*4*) have shown that the average percentage of eosinophiles in the blood of normal cattle is 13.15. Whatever the cause may be, it certainly presents a very interesting and important problem for further research. According to the tables given by Burnett (*2*) the percentages of eosinophiles are about the same for all animals excepting cattle.

One thing that adds difficulty to the study of the blood in apparently normal animals is that nearly all are harboring more or less large numbers of parasites of various genera. Two apparently normal animals observed have presented a very high percentage of eosinophiles. No. 8 for nine examinations over a period of six months averaged 17.2 per cent of eosinophiles. On autopsy, this animal showed a relatively small number of internal parasites. No. 1 for one examination showed 13 per cent, and on autopsy there were found large numbers of internal parasites.

As these were the only two animals that showed unusually large numbers of eosinophiles, very little more can be said about their presence as an aid to the diagnosis of internal parasites. One examination can be picked from some of the other cases that shows nearly as high a percentage as did No. 1. Again, it can be said that results will be conclusive only as the observations are made over a period of time on the same animal. Then also, one must consider that for some cause the animals might become more severely infested with parasites, or else they might get rid of them. There is apparently some seasonal variation, especially in regard to the intestinal worms, more being present in the fall and winter than in the spring and summer. This also should be considered.

[1] Reference is made by number (italic) to "Literature cited," p. 688.

TABLE II.—*Blood counts of horses in poor condition due to internal parasites*

Tag No.	Date.	Hemoglobin.	Erythrocytes.	Leucocytes.	Lymphocytes.	Polymorphonuclears.	Mononuclears.	Eosinophiles.	Mast cells.	Transitionals.
	1918.									
A....	Sept. 11	70	3,888,000	6,333	32.6	57.8	5.4	3.4	0.8	0
	Oct. 15	62	5,120,000	7,833	31.0	62.6	6.0	.4	0	0
B....	Oct. 19	63	5,880,000	9,766	33.4	58.2	4.6	3.6	.2	0
	Oct. 29	60	5,608,000	7,291	31.4	58.4	8.4	1.6	.2	0
C.....	Sept. 11	80	6,424,000	7,333	28.4	60.2	7.2	4.2	0	0
	Oct. 1	.....	5,352,000	7,555	32.4	57.2	9.0	1.4	0	0
E....	Sept. 11	60	8,640,000	8,342	30.6	41.2	27.0	1.2	0	0
	Oct. 23	90	7,232,000	6,773	28.8	62.4	5.8	3.0	0	0
	Oct. 29	70	6,980,000	9,639	42.6	33.0	18.6	5.8	0	0
F....	Sept. 11	80	6,432,000	7,963	43.6	39.4	15.2	1.6	.2	0
	Oct. 19	60	4,400,000	6,839	36.2	70.2	3.6	0	0	0
	Oct. 21	63	5,568,000	13,867	30.8	58.6	7.0	3.6	0	0
G....	Sept. 12	70	4,524,000	8,327	32.8	52.4	12.0	2.8	0	0
	Oct. 18	60	4,634,000	4,837	24.4	67.2	7.8	.6	0	0
H....	Sept. 12	80	5,728,000	11,149	26.4	42.0	28.6	3.0	0	0
	Oct. 19	53	4,288,000	7,983	39.8	54.8	3.6	1.8	0	0
D....	Sept. 11	70	6,080,000	12,642	33.8	52.2	10.6	3.4	0	0
	Oct. 23	76	6,832,000	7,637	24.6	58.0	16.0	1.2	.2	0
	Oct. 29	80	7,008,000	6,890	45.2	32.6	22.0	.2	0	0
	Average.	69.2	5,822,000	8,363	33.1	53.6	11.4	2.2	.08	0

A.—Three-year-old gelding with Strongylosis.

At autopsy October 15, 1918, there were found about 150 *Gastrophilus* spp. in the pylorus, a serious infestation of Strongylus and Cylicostomes in the cecum and colon, and a small aneurism of the anterior mesentery artery containing live embryos.

B.—Four-year-old gelding with Strongylosis.

At autopsy December 17, 1918, there were found about 100 Gastrophilus with Strongylus and Cylicostomes.

C.—Four-year-old mare with Strongylosis.

At autopsy, there were found 14 *Ascaris equorum* in the duodenum and a moderately severe infestation of the cecum and colon with Strongylus and Cylicostomes.

E.—Three-year-old gelding with Strongylosis.

At autopsy, there was found a severe infestation of *Gastrophilus* spp. in the pylorus and a very severe infestation of Strongylus and Cylicostomes in the cecum and colon.

F.—Four-year-old mare with Strongylosis.

At autopsy, October 21, 1918, there were found a few *Gastrophilus* spp. in the pylorus. There were 18 *Ascaris equorum* in the duodenum, 5 *Setaria equina* and 20 Strongylus in the abdominal cavity, and a very severe infestation of Strongylus and Cylicostomes in the cecum and colon.

G.—Four-year-old mare with Strongylosis.

At autopsy, October 18, 1918, there were found a moderate number of *Gastrophilus* spp. in the pylorus, 2 Ascares in the duodenum, 1 Strongylus and 10 Setaria in the abdominal cavity, and a very severe infestation of Strongylus and Cylicostomes in the cecum and colon.

H.—Three-year-old gelding with Strongylosis.

At autopsy, on October 24, 1918, there was found a moderate number of *Gastrophilus* spp. in the pylorus, one Strongylus and four Setaria in the abdominal cavity, and a very severe infestation of Strongylus and Cylicostomes in the cecum and colon.

D.—Three-year-old gelding in very poor condition, which, as far as could be ascertained, was due to the *Gastrophilus* spp.

At autopsy, there were found 1,030 *Gastrophilus* spp. in the pylorus, 12 Strongylus in the cecum and colon, and 3 Setaria in the abdominal cavity.

These cases are the ones that prompted this report. One of the main features of the positive diagnosis of internal parasitic diseases of man and the domestic animals apparently has been the presence of an eosinophilia.

On this basis, there being an absence of an eosinophilia and the animals having a high temperature, the cases were diagnosed as infectious equine anemia. The symptoms of the two diseases are very similar. Transmission experiments failed in a large number of cases, and, therefore, infectious equine anemia can be positively excluded. In a few months all the animals died or were killed. Seven of the animals had very severe infestations of Strongylus and Cylicostomes; the eighth harbored an enormous number of *Gastrophilus* spp.

At the time of the first examination the animals were not in bad physical condition. Some, in fact, were in good physical condition. Their resistance should have been good. From the facts presented by these cases it can be seen that other factors than the presence or absence of an eosinophilia are of great importance in the diagnosis of internal parasites of the horse.

TABLE III.—*Miscellaneous blood counts*

Tag No.	Date.	Hemoglobin.	Erythrocytes.	Leucocytes.	Lymphocytes.	Polymorphonuclears.	Mononuclears.	Eosinophiles.	Mast cells.	Transitionals.
12..	Feb. 2, 1919	90	7,642,000	11,420	39.2	49.2	7.4	4.2	0.0	0
	Mar. 8, 1919	90	7,964,000	7,039	34.2	60.2	2.4	3.2	0	0
	Mar. 27, 1919	90	6,836,000	7,200	32.0	60.0	7.4	.6	0	0
	Apr. 26, 1919	100	8,076,000	9,389	33.2	58.6	6.0	2.2	0	0
	May 16, 1919	60	5,864,000	7,349	39.2	55.6	6.4	.8	0	0
10..	Dec. 22, 1919	85	7,962,000	5,369	44.0	44.4	9.0	2.4	.2	0
	Jan. 3, 1919	80	6,934,000	5,387	10.6	82.4	6.0	1.0	0	0
	Average.....	85.0	7,325,400	6,693	33.2	58.6	6.3	2.0	.02	0

No. 12.—Twelve-year-old animal that apparently died from infectious equine anemia; positive diagnosis doubtful.

At autopsy, on May 24, 1919, there were found about 150 *Gastrophilus* spp., a moderate infestation of the cecum and colon with Strongylus and Cylicostomes, and a small aneurism of the anterior mesenteric artery containing a few live embryos.

No. 10.—Aged, healthy animal at time of first blood count; at the last the animal was very ill with septicemia.

At autopsy, on January 8, 1919, there were found 35 *Gastrophilus* spp., 2 Setaria in the abdominal cavity, and a few Strongylus and Cylicostomes in the cecum and colon.

TABLE IV.—*Blood counts of horses with acute cases of infectious equine anemia*

Tag No.	Date.	Hemoglobin.	Erythrocytes.	Leucocytes.	Lymphocytes.	Polymorphonuclears.	Mononuclears.	Eosinophiles.	Mast cells.	Transitionals.
11..	Mar. 1, 1919	80	6,934,000	4,360	34.2	53.0	3.6	9.0	0.2	0
	Mar. 8, 1919	80	8,132,000	7,261	21.2	76.8	2.0	0	0	0
6...	Dec. 16, 1918	100	8,320,000	7,341	33.0	57.2	5.8	4.0	0	0
	Jan. 3, 1919	100	7,366,000	7,639	27.4	61.2	8.2	3.2	0	0
	Mar. 6, 1919	100	7,932,000	5,482	28.2	56.2	9.0	6.6	0	0
	Mar. 25, 1919	80	7,629,000	9,870	49.2	41.4	7.2	2.2	0	0
	Mar. 27, 1919	80	7,368,000	7,296	20.6	72.4	3.6	3.2	.2	0
	Apr. 8, 1919	80	6,884,000	7,329	22.4	73.6	3.6	.4	0	0
M..	Nov. 7, 1918	20	1,856,000	4,683	30.6	30.2	3.6	0	0	0
N..	Nov. 7, 1918	70	3,824,000	6,281	39.6	28.4	9.2	1.0	.4	31.4
L...	Nov. 16, 1918	50	4,864,000	9,669	29.0	36.7	3.3	1.0	0	30.0
758.	Nov. 7, 1918	80	4,320,000	6,993	22.6	66.2	9.2	2.0	0	0
	Oct. 8, 1918	100	8,634,000	6,981	26.6	63.0	4.6	3.8	0	0
	Nov. 2, 1918	90	7,824,000	9,873	33.6	61.0	3.2	2.2	0	0
	Nov. 14, 1918	60	6,408,000	6,321	14.2	82.2	3.6	0	0	0
	Nov. 15, 1918	70	6,488,000	7,369	23.6	71.4	4.2	.8	0	0
K..	June 2, 1919	50	4,872,000	6,329	35.4	60.2	4.2	.2	0	0
	June 17, 1919	30	3,486,000	5,317	28.4	57.4	11.2	3.0	0	0
J...	Mar. 26, 1919	80	.....	.....	37.8	55.4	2.6	4.2	0	0
	Average.....	73.6	6,285,600	7,021	29.3	58.1	5.4	2.5	.04	3.3

No. 11.—Aged animal with an acute experimental case of infectious equine anemia; inoculated February 19, 1919, and died March 23, 1919.

At autopsy, there were found about 100 *Gastrophilus* spp. in the pylorus, 1 Setaria in the abdominal cavity, and a moderate infestation of the cecum and colon with Strongylus and Cylicostomes.

No. 6.—Eight-year-old gelding with an acute experimental case of infectious equine anemia injected March 6, 1919.

At autopsy on March 10, 1919, there were found about 30 *Gastrophilus* spp. and 25 *Setaria equina*.

M.—Four-year-old mare with an acute case of infectious equine anemia.

At autopsy, on November 9, 1919, there were found a few *Gastrophilus* spp., one Setaria, a small worm-free aneurism of the anterior mesenteric

artery, and an enormous infestation of the cecum and colon with Strongylus and Cylicostomes.

N.—Ten-year-old mare with an acute case of infectious equine anemia.

At autopsy, on November 26, 1918, there were found a few *Gastrophilus* spp., eight *Ascaris equorum*, five Setaria, a few Strongylus and Cylicostomes in the cecum and colon, and a small anuerism of the anterior mesenteric artery containing live embryos.

L.—Aged mare with an acute case of infectious equine anemia.

At autopsy, December 24, 1918, there were found a few *Gastrophylus* spp. and a serious infestation of Strongylus and Cylicostomes in the cecum and colon.

No. 758.—Middle-aged gelding with an acute case of infectious equine anemia.

At autopsy, on November 17, 1918, there was found one Setaria in the abdominal cavity.

K.—Five-year-old gelding with an acute case of infectious equine anemia.

At autopsy, on June 17, 1919, there were found about 50 *Gastrophylus* spp., a small worm-free aneurism of the anterior mesenteric artery, and a severe infestation of the cecum and colon with Strongylus and Cylicostomes.

J.—Aged 7-year-old gelding with an acute case of infectious equine anemia.

At autopsy, on March 26, 1919, there were found 50 *Gastrophilus* spp., a few Strongylus and Cylicostomes in the cecum and colon, about 60 Setaria in the abdominal cavity, and an enormous aneurism of the anterior mesenteric artery containing large numbers of live embryos.

TABLE V.—*Blood counts of horses with subacute cases of infectious equine anemia*

Tag No.	Date.	Hemoglobin.	Erythrocytes.	Leucocytes.	Lymphocytes.	Polymorphonuclears.	Mononuclears.	Eosinophiles.	Mast cells.	Transitionals.
1	Aug. 17, 1917...	45	1,940,000	1,888	48.9	46.6	3.7	0.2	0.6	0
21	June 9, 1919...	80	6,974,000	8,876	25.6	65.0	7.2	2.2	0	0
	July 3, 1919...	90	7,242,000	8,693	29.0	55.6	4.2	1.2	0	0
	Sept. 12, 1919...	80	6,464,000	7,560	37.6	52.2	9.0	1.2	0	0
	Average.	73.8	5,655,000	6,754	37.8	54.9	6.0	1.2	.15	0

No. 1.—At the time this blood examination was made the animal was in the early stage of a subacute case of infectious equine anemia.

At autopsy, August 21, 1917, there were found a few *Gastrophilus* spp. and a moderate infestation and Strongylus and Cylicostomes in the cecum and colon.

No. 21.—Aged mare with a subacute case of infectious equine anemia. At autopsy, on September 29, 1919, there were found severe infestations of Strongylus and Cylicostomes in the cecum and colon.

TABLE VI.—*Blood counts of horses with chronic cases of infectious equine anemia*

Tag No.	Date.	Hemoglobin.	Erithrocytes.	Leucocytes.	Lymphocytes.	Polymorphonuclears.	Mononuclears.	Eosinophiles.	Mast cells.	Transitionals.
	Nov. 27, 1917	110	8,120,000	18,222	17.2	76.8	6.0	0	0	0
	Dec. 15, 1917	90	7,456,000	9,650	35.8	44.2	15.0	5.0	0	0
	May 2, 1918	89	7,728,000	8,200	30.4	61.6	7.6	1.2	.2	0
	May 16, 1918	75	7,422,000	10,200	32.6	60.2	5.0	2.2	0	0
	June 14, 1918	90	9,620,000	800	41.8	47.4	7.4	2.4	1.0	0
671	June 18, 1918	100	7,898,000	10,320	28.4	61.0	6.2	3.4	1.0	0
	June 24, 1918	78	9,080,000	5,310	36.2	55.6	3.8	4.0	.4	0
	Aug. 5, 1918	110	7,892,000	11,700	37.2	44.6	11.0	6.4	.8	0
	Aug. 26, 1918	87	8,072,000	7,633	37.0	48.6	7.4	6.2	.8	0
	Oct. 10, 1918	86	7,138,000	7,963	28.0	57.6	12.2	2.2	0	0
	Oct. 29, 1918	70	6,408,000	8,888	30.4	61.2	6.4	2.0	0	0
	Nov. 3, 1918	40	3,936,000	5,183	21.6	73.0	4.2	1.2	0	0
	Oct. 10, 1918	90	7,638,000	7,383	38.6	51.8	5.2	4.4	0	0
	Nov. 2, 1918	90	6,938,000	8,373	25.0	67.8	6.4	.8	0	0
	Jan. 2, 1919	80	4,864,000	7,639	33.4	57.2	8.0	1.4	0	0
	Jan. 11, 1919	80	5,836,000	7,939	33.0	52.2	9.2	5.6	0	0
	Jan. 28, 1919	40	5,120,000	7,631	33.6	58.0	7.0	1.4	0	0
	Mar. 13, 1919	90	7,264,000	7,360	16.0	77.2	6.4	.4	0	0
753	Mar. 19, 1919	80	5,872,000	8,390	54.4	42.6	3.0	0	0	0
	Mar. 25, 1919	70	5,984,000	6,390	53.6	42.6	1.2	2.6	0	0
	Apr. 26, 1919	80	6,984,000	5,387	37.4	58.2	3.6	.8	0	0
	May 16, 1919	90	7,638,000	5,931	34.2	51.2	9.4	5.2	0	0
	June 9, 1919	100	7,764,000	6,390	33.6	60.4	3.2	2.8	0	0
	July 3, 1919	100	7,634,000	7,961	38.6	57.2	4.4	.8	0	0
	Sept. 12, 1919	90	7,688,000	7,833	49.2	42.8	3.8	4.2	0	0
	May 2, 1918	74	8,032,000	7,300	41.6	50.2	4.8	2.8	.6	0
	May 11, 1918	85	6,848,000	7,480	28.6	61.4	4.4	5.6	0	0
	May 14, 1918	90	.....	.....	47.0	41.6	8.2	3.0	.2	0
	May 20, 1918	90	7,136,000	8,000	12.6	79.2	4.0	1.4	2.8	0
	May 23, 1918	90	7,840,000	7,333	29.4	67.2	4.8	.6	0	0
	May 28, 1918	95	7,232,000	8,000	43.6	35.0	16.8	2.2	2.2	0
672	June 3, 1918	90	7,326,000	7,960	33.0	58.2	4.4	4.2	.2	0
	June 12, 1918	77	5,184,000	6,120	41.6	45.4	8.4	3.6	1.0	0
	June 18, 1918	86	6,912,000	5,580	25.6	57.2	14.8	2.0	.4	0
	June 24, 1918	84	7,612,000	3,330	32.6	53.0	10.0	3.8	.6	0
	July 5, 1918	95	7,642,000	6,410	28.8	60.4	5.8	5.0	0	0
	July 8, 1918	68	6,080,000	7,200	28.2	63.4	4.4	3.0	1.0	0
	July 13, 1918	78	6,368,000	7,200	12.4	74.4	13.2	0	.4	0
24	Sept. 18, 1919	90	6,988,000	5,363	27.0	65.0	5.4	2.6	0	0
	Nov. 1, 1919	80	6,464,000	6,666	25.4	57.4	13.6	1.0	2.6	0
25	Dec. 24, 1917	80	7,264,000	6,733	30.2	49.4	13.6	6.8	0	0
	Mar. 18, 1920	100	8,328,000	4,328	No count made.					
	June 4, 1919	100	7,856,000	4,960	32.6	61.2	4.8	1.4	0	0
	July 3, 1919	80	6,398,000	7,333	36.6	53.8	8.2	1.4	0	0
18	Aug. 25, 1919	80	7,288,000	6,366	40.2	46.2	6.0	7.6	0	0
	Sept. 12, 1919	80	6,972,000	4,888	40.2	49.0	6.2	4.6	0	0
	Nov. 1, 1919	90	8,262,000	9,363	38.4	51.4	9.6	1.2	0	0
	Dec. 24, 1919	80	7,074,000	5,366	38.3	54.6	6.1	1.0	0	0
	Aug. 23, 1919	90	7,844,000	7,888	29.0	60.2	4.4	6.0	0	0
	Sept. 12, 1919	70	6,864,000	7,391	21.2	68.2	5.4	5.2	0	0
23	Oct. 9, 1919	60	5,834,000	7,200	42.2	47.2	6.4	4.2	0	0
	Nov. 1, 1919	80	6,824,000	4,266	39.4	44.4	13.0	3.2	0	0
	Dec. 24, 1919	70	6,876,000	4,866	40.4	46.2	11.0	2.4	0	0
	Average.....	84.0	7,119,000	7,225	33.5	56.0	7.3	2.9	.30	0

No. 671.—Aged gelding with a chronic case of infectious equine anemia.

At autopsy on November 4, 1918, there were found a few Strongylus and Cylicostomes in the cecum and colon and a small aneurism of the anterior mesenteric artery containing one live embryo.

No. 753.—Four-year-old colt with a chronic case of infectious equine anemia.

At autopsy there were found a moderate number of Strongylus and Cylicostomes in the cecum and colon and three *Setaria equina* in the abdominal cavity.

No. 672.—Middle-aged mare with a chronic case of infectious equine anemia.

At autopsy on July 14, 1918, there were found small numbers of *Gastrophilus* spp. and a moderate infestation of the cecum and colon with Strongylus and Cylicostomes.

No. 24.—Aged animal with a chronic case of infectious equine anemia.

At autopsy November 14, 1919, many *Gastrophilus* spp. were found, 10 Setaria, a serious infestation of Strongylus and Cylicostomes in the cecum and colon, and a small aneurism of the anterior mesenteric artery containing live embryos.

No. 25.—Twelve-year-old mare with a chronic case of infectious equine anemia.

At autopsy March 18, 1920, there were found a few *Gastrophilus* spp. and a moderate number of Strongylus and Cylicostomes in the cecum and colon.

No. 18.—Ten-year-old gelding with a chronic case of infectious equine anemia.

At autopsy on February 10, 1920, there were found three *Gastrophilus* spp., a few Strongylus and Cylicostomes, and a small worm-free anuerism of the anterior mesenteric artery.

No. 23.—Aged gelding with a chronic case of infectious equine anemia.

At autopsy on February 17, 1920, there were found about 150 *Gastrophilus* spp. and a moderate number of Strongylus and Cylicostomes in the cecum and colon.

Hadwen (7) has noted that the eosinophiles in cases of swamp fever (infectious equine anemia) are diminished in number and sometimes absent from the peripheral blood. In the cases here reported this is noticeable more especially in the acute cases than the chronic ones. There is very little, if any, decrease in the number of the eosinophiles in some of the more chronic cases.

In passing, it may well be said that in connection with some parasitic anaphylaxis experiments injection of ground up *Gastrophilus* spp. produced a marked local eosinophilia with severe abscess development. Local injection of ground up Strongylus produced a mild abscess

formation with practically no local eosinophilia. Mention of this is made in the hope that further observations will be made.

The work of Bücklers (*1*), Deglos (*3*), Giffin (*5*), Hadwen (*6*), Moore, Haring, and Cady (*8*), and Nazum (*9*) should also be consulted in connection with the study of eosinophilia.

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A SOURING OF BEEF CAUSED BY BACILLUS MEGATHERIUM

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The problem involved in the maintenance of a wholesome food supply for the public is without doubt one of paramount importance, concerning, as it does most intimately, the physical well-being of the Nation. Among the most difficult phases of this problem is that respecting the flesh foods. The long recognized fact of the communicability to man of certain animal diseases has made it imperative for the safeguarding of health that supervision be maintained over the extremely important industries engaged in the production of flesh foods.

But there are other considerations which stress the urgency of a proper watch over the character of the flesh foods to be offered to the people for consumption. Meats, to be acceptable to man, must be not only wholesome but palatable. It is quite conceivable that the flesh of a carcass which has satisfactorily passed a rigid inspection as to the existence of disease may subsequently be subjected to such a manner of handling as to render it utterly unsuitable for food. This is what actually occurs at times.

Aside from the objectionable qualities which may be normal or incidental to animals presented for slaughter, such as the sexual odors and flavors of certain adult male animals or odors and flavors produced by the use of ill-smelling feeds, etc., the flesh of animals from the time of slaughter to the time of consumption is continually susceptible to the acquisition of undesirable properties. Some of these may be acquired by absorption and others by the action of bacteria, as in ordinary putrefaction.

There is a condition known as sour beef, which is familiar alike to butchers and inspectors, and one which doubtless occasions no little economic loss, especially to small butchering establishments and retailers who may not be equipped with facilities for the storage of meat to prevent this alteration. In reference to this condition the following statement is quoted from Ostertag's "Handbook of Meat Inspection":[1]

> Stinking acid fermentation occurs in slaughtered domestic animals when the meat, while still warm, is stored in large pieces and in closed receptacles, or, in general, when it is subjected to conditions under which it can not cool. This alteration is characterized by the term "suffocated."

[1] OSTERTAG, Robert. HANDBOOK OF MEAT INSPECTION . . . Translation by Earley Vernon Wilcox. ed. 2, rev., p. 746–747. New York. 1905.

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It is not definitely known that the souring of beef referred to by Ostertag is identical with that which is discussed in this study.

Certainly the conservation of the interests of the consuming public in this regard can be accomplished only by precautions of a far-reaching nature, involving vigilance from the abattoir to the platter.

The common stock of knowledge as to the definite cause of the phenomenon known as sour beef is probably more or less vague.

A specimen of sour beef was sent to this laboratory from one of the large packing establishments with a view to determining definitely the cause of the condition in this specific instance, as the meat was very decidedly sour. The specimen was assigned the number 1510.

BACTERIOLOGICAL EXAMINATION OF SOUR BEEF

An effort was made to isolate any organisms that might be found in the interior of the specimen. This was accomplished in the following manner: A site was selected favorable to making a deep cut into the body of the specimen, and the surface was then seared for a radius of several inches with a hot platinum spatula. With a sterile scalpel and forceps this surface was removed to a depth of ½ inch. The newly exposed surface was then similarly seared, and with the aid of a fresh sterile scalpel and forceps a small cube of meat was aseptically taken out and placed into a tube of bouillon medium.

This bouillon culture showed appreciable growth at the end of 24 hours' incubation at 37° C. It was then shaken thoroughly, and a loopful of the suspension of organisms was distributed upon the surface of each of three plain agar plates.

Upon incubation for 24 hours at 37° C. these plates developed three types of organisms, which were later transplanted upon plain agar slants, and for convenience of identification designated as organism 1510–A, 1510–B, and 1510–C, respectively. Organism 1510–A proved to be *Staphylococcus albus*. Organism 1510–B was a medium short chain-forming and apparently spore-bearing bacillus. Organism 1510–C was a fungus.

An effort was then made to determine whether any one of these three organisms or any combinations thereof would artificially produce the sour-beef odor in meat as noticed in the original specimen. Sterile normal beef muscle pieces were placed in sterile plugged test tubes, and the following tests were applied.

Two tubes were inoculated with each organism and two tubes with each combination of two organisms. All of the tubes were incubated for 24 hours at 37° C., with the following results:

Organism 1510–A produced no odor.

Organism 1510–B produced a distinct sour-beef odor.

Organism 1510–C produced a musty, fungous odor.

Organisms 1510–A and B together produced a slight sour-beef odor.

Organisms 1510–A and C together produced a slight musty odor.

Organisms 1510–B and C together produced a blending of sour-beef and musty odors.

All of the meat pieces which were inoculated with the fungus were covered with a heavy overgrowth of this organism.

This experiment seemed to fix the responsibility for the souring of this specimen of beef upon organism 1510–B independently of the other two organisms. Both organisms 1510–A and 1510–C will therefore be

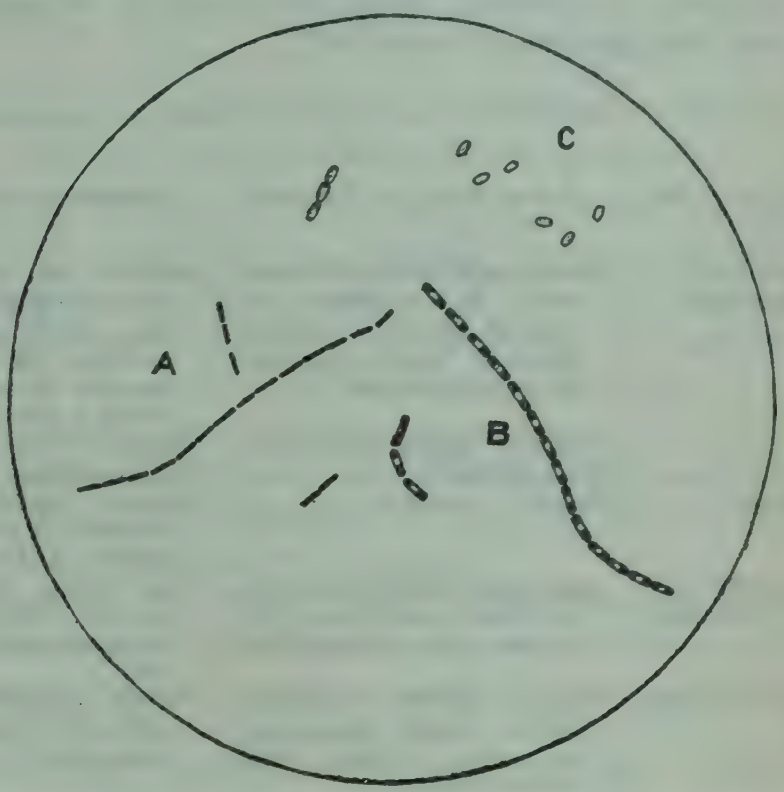

FIG. 1.—A, chains of the vegetative form of the organism; B, chains of organisms in beginning sporulation forms, containing immature spores; C, completely formed spores, in which stage the chain formation is invariably lost. Pen-and-ink drawing from microscope with aid of camera lucida.

disregarded from this point as incidental and of no significance in the souring of beef.

DESCRIPTION OF CAUSATIVE ORGANISM

Various diagnostic media were then employed to determine if possible the identity of organism 1510–B. It was found that the morphology and cultural characteristics of the organism corresponded more closely to those of *Bacillus megatherium* var. *Ravenellii* and *B. megatherium* var. *de Bary*, as described by Chester,[1] than to any other organism described.

The organism 1510–B may be briefly described as follows:

A round-ended, chain-forming, spore-bearing, Gram-positive, aerobic, slightly motile rod, from 2 to 5 microns long and 2 microns wide. During

[1] CHESTER, Frederick D. A MANUAL OF DETERMINATIVE BACTERIOLOGY. p. 271–272, 277. New York, London. 1909.

sporulation the organisms become symmetrically swollen, and the chains, naturally fragile, become even more so. The spores are equatorial. Figure 1 shows the microscopic appearance of organism 1510–B in the vegetative form (A) and in the immature (B) and mature (C) stages of sporulation. Isolated 24-hour colonies when slightly magnified are seen to be densely opaque at the center. The periphery is granular, with the the appearance of irregular circles composed of rhomboidal rugae.

In Table I there is given a comparative digest of the cultural characteristics of *Bacillus megatherium* var. *de Bary* and *B. megatherium* var. *Ravenellii*, as given by Chester,[1] along with those of organism 1510–B, recovered from the specimen of sour beef.

TABLE I.—*Comparison of cultural characteristics of organisms*

B. megatherium var. *de Bary.*	*B. megatherium* var. *Ravenellii.*	Organism 1510–B of sour beef.
Aerobe, flagellated, 2 to 5 microns wide.	Aerobe and facultative anaerobe. Rod-rounded, 3 to 5 times breadth. Chain-forming, spore-bearing. Rods not swollen.	Aerobic rod, rounded ends, chain-forming, spore-bearing. Rods symmetrically swollen at sporulation. Length 2 to 5 microns, width 2 microns.
	Slightly motile, ameboid...	Slightly motile.
Gram-positive.....	Gram-positive.....	Gram-positive.
Gelatin liquefied.....	Gelatin liquefied.....	Gelatin liquefied. No gas fermentation in dextrose, lactose, and saccharose.
Agar slant growth like *B. subtilis;* yellowish.	Agar slant, white, glistening, elevated, sometimes yellowish, odor of sour milk.	Agar slant, smooth, dull, grayish white, luxuriant in 24 hours at 37° C. Sour odor.
Potato, like *B. subtilis;* yellowish.	Potato, growth elevated, white, moist, glistening, spreading, rugose. Odor of stale milk.	Potato, smooth, glistening, yellowish white, moist, but scant growth.
	Litmus milk decolorized, slowly peptonized. Amphoteric. Alkaline.	Litmus milk coagulated, peptonized, decolorized. Neutral to P_H.
Indol negative, hydrogen sulphid (H_2S) positive.	Indol negative.....	Indol negative.
Habitat, cabbage infusion.	Habitat, soil.....	Habitat, sour beef.

Fermentation reactions as shown in Table II were obtained after 60 hours' incubation with *Bacillus megatherium* var. *de Bary* and organism 1510–B.

[1] CHESTER, Frederick D. OP. CIT.

TABLE II.—*Comparison of fermentation reactions*

Organism.	Dextrose.	Lactose.	Saccharose.	Mannite.	Xylose.	Levulose.
B. megatherium	Acid	Negative.	Slight acid.	Negative.	Negative.	Acid.
1510–B	do	do	Acid	do	do	Do.

Organism.	Galactose.	Inulin.	Arabinose.	Dulcite.	Maltose.	Salacin.
B. megatherium	Acid	Negative.	Negative.	Negative.	Acid	Acid.
1510–B	do	do	do	do	do	Negative.

CHEMICAL EXAMINATION OF SOUR BEEF

To determine the identity of the acid produced by organism 1510–B in the process of souring beef, the following preparations were made:

About 125 gm. of sterile raw meat pieces were inoculated with organism 1510–B and incubated for eight days. A corresponding container of sterile raw beef, uninoculated, was likewise treated as a control. At the end of eight days both specimens were removed from the incubator.

The inoculated specimen had acquired an intensely sour odor, characteristic of that previously produced by the organism in meat, while the control specimen was apparently unchanged.

As a control on the purity of the culture which had produced the souring, bouillon cultures were inoculated with fluids from the sour specimen. These cultures were plated, and eight representative colonies were "picked off" from the plates, each of which colonies subsequently developed, on agar slants, growths which invariably were true to type in morphology, cultural characteristics, and the ability to reproduce sour beef.

It was therefore concluded that this specimen was rendered sour by a pure culture of organism 1510–B.

This specimen and the uninoculated control specimen were then submitted to J. F. Couch, pharmacological chemist of the Pathological Division, Bureau of Animal Industry, for an identification of the acid produced in the meat by the organism 1510–B.

The report of his technic is as follows:

Both samples were cooled in a refrigerator and were then extracted with cold ether. Upon evaporation of the ether extracts the residue from the normal beef deposited a small quantity of fatty matter which was neutral in reaction and had the odor of beef tallow. The residue obtained from the inoculated beef, however, was acid to litmus and carried the offensive putrefaction odor of the sour-beef specimen. From this small residue some evidence of propionic acid was obtained. The sour-beef specimen was, therefore, extracted with 300 milliliters of 2 per cent sulphuric-acid solution by digestion at a moderate temperature. The aqueous solution was filtered by suction, and the clear filtrate, which had the characteristic putrefaction odor, was

submitted to steam distillation. The distillate was acid and carried the putrefaction odor. This was titrated against *N*/5 sodium hydroxid, using phenolphthalein as indicator. Neutralization required 50.78 milliliters. The solution of the sodium salt was then concentrated by distilling off the solvent. The distillate carried the putrefaction odor and was neutral to litmus. The residue was transferred to a glass evaporating dish, dried to constant weight, at 105° C. and weighed. Weight of sodium salt, 0.9704 gm. Calculated for $C_2H_5CO_2Na$, 0.9855 gm. The identity of the acid was confirmed by conversion into the barium and silver salts, by solubility and organoleptic tests, and by Dyer's color test.[1]

PATHOGENICITY AND TOXIN PRODUCTION

Tests were conducted to determine the pathogenicity of the organism recovered from sour beef and to ascertain whether it produces toxin in meat or on artificial media.

Two guinea pigs were inoculated with a normal saline suspension of a culture of the organism taken from a plain agar slant, one guinea pig receiving the inoculation subcutaneously and the other intraperitoneally. No ill effects were produced by this organism.

A third guinea pig was fed pieces of meat thoroughly soured with the organism and was also "drenched" with a heavy saline suspension of the organisms. This animal lived.

Two guinea pigs were inoculated intraperitoneally each with 1 cc. of a heavy saline suspension of the washed organisms from a 24-hour agar culture. They were not affected.

Two guinea pigs were inoculated intraperitoneally with the supernatant fluid of a 24-hour bouillon culture of the organism but showed no ill effects.

These tests indicate that this organism is not pathogenic for guinea pigs and does not produce any appreciable toxin in ordinary media or raw beef under laboratory conditions.

COMPARATIVE ODOR PRODUCTION OF OTHER ORGANISMS

An experiment was conducted to compare the odor produced on meat by this organism with any odors that might be produced by other organisms selected at random. Sterile normal meat pieces were inoculated with various organisms in order to determine whether this characteristic sour-beef odor would be reproduced or approximated by any of them on raw meat. These inoculated meat pieces were incubated for four days at 37° C. The following organisms failed to produce any perceptible odor:

Bacillus pyocyaneus.
Bacillus of Priesz and Nocard.
Staphylococcus albus.
Staphylococcus citreus.

[1] DYER, D. C. A NEW METHOD OF STEAM DISTILLATION FOR THE DETERMINATION OF THE VOLATILE FATTY ACIDS, INCLUDING A SERIES OF COLORIMETRIC QUALITATIVE REACTIONS FOR THEIR IDENTIFICATION. In Jour. Biol. Chem., v. 28, no. 2, p. 469. 1917.

B. bronchisepticus.
B. typhi murium.
Actinomyces bovis.
Moeller's grass bacillus.
B. lactimorbis.
B. pullorum.
B. mesentericus.
B. prodigiosus.
B. enteritidis.
B. coli communis.
B. coli communior.
B. icteroides.
Streptococcus mastitis.
B. gallinarium.
B. subtilis.

The following organisms produced odors as described:

Saccharomyces, slight sweetish odor.

Blastomyces, distinct yeasty odor.

Bacillus vulgatus, chestnut odor.

Organism 1510–B again produced the typical sour-beef odor.

The odor produced by organism 1510–B is strikingly characteristic; it is readily distinguishable from the yeasty or putrefactive odors, and it is not produced on raw beef by any of the other organisms used in this experiment.

THERMAL AND OXYGEN REQUIREMENTS

A facultative test of the beef-souring property of organism 1510–B with regard to temperature and oxygen tension was next applied. Meat pieces were inoculated with the organism and treated as follows for four days, with the results noted in each case:

(1) Aerobically at room temperature. Produced perceptible sour-beef odor, though not so strong as when incubated.

(2) Aerobically in refrigerator (0° to 4.5° C.). Produced a faint suggestion of sour-beef odor.

(3) Anaerobically in incubator. Produced no odor.

(4) Under partially reduced oxygen tension in incubator. No odor.

It was concluded from these results that the organism 1510–B is an aerobic saprophyte with a wide range of vegetative temperatures but with an optimum of about 37° C.

SEROLOGY

It was then decided to determine if possible the existence of a specific or group agglutination reaction which would establish a relationship between the organism isolated from sour beef and *Bacillus megatherium.*

Cultures of *Bacillus megatherium* No. 270 and 734 were obtained from the American Museum of Natural History of New York City. No. 734 was the de Bary strain, but the identity of No. 270 was unknown.

Two rabbits were hyperimmunized against organism 1510–B by intravenous injections of 0.5 cc. of a washed saline suspension of the organism on the first day, 1 cc. on the fifth day, 2 cc. on the twelfth day, and 2 cc. on the nineteenth day. Samples of blood serum were then taken and were tested against carbolized normal salt solution suspensions of the organisms (agglutinating fluids). A sample of normal rabbit serum was also tested against each organism as a control. After 24 hours' incubation at 37° C, the tests were read, as shown in Table III.

TABLE III.—*Agglutination tests*

Animal.	*B. megatherium*, 270.					
	0.04	0.02	0.01	0.005	0.002	0.001
Hyperimmune rabbit A	+	+	+	+	?	?
Hyperimmune rabbit B	+	+	+	+	+	+
Normal rabbit C	?	?	−	−	−	−

Animal.	*B. megatherium*, var. *de Bary*, 734.					
	0.04	0.02	0.01	0.005	0.002	0.001
Hyperimmune rabbit A	+	+	+	+	?	−
Hyperimmune rabbit B	+	+	+	+	−	−
Normal rabbit C	−	−	−	−	−	−

Animal.	Organism 1510–B.					
	0.04	0.02	0.01	0.005	0.002	0.001
Hyperimmune rabbit A	+	+	+	+	+	+
Hyperimmune rabbit B	+	+	+	+	+	+
Normal rabbit C	−	−	−	−	−	−

Anaerobic cultures of the three organisms on 3 per cent glycerin agar were incubated 18 hours at 37° C. with no apparent growth. At 60 hours' incubation these cultures all show minute dewdrop colonies. The same cultures were then incubated aerobically for 24 hours at 37°, producing a luxuriant growth, typical of *Bacillus megatherium*.

The apparent identity of the organism 1510–B as *Bacillus megatherium* now appeared to be clearly established by its morphological and cultural likeness to the known organism of that name. It remained to be seen, however, whether the phenomenon of the souring of beef could be reproduced by a known culture of *B. megatherium* derived from a

source other than sour beef. For a determination of this problem both cultures obtained from the American Museum of Natural History were used for the inoculation of sterile specimens of raw beef. A third specimen was inoculated with organism 1510–B. After 24 hours' incubation it was found that the typical odor of sour beef had become pronounced in each of the samples inoculated. It was therefore concluded that *B. megatherium* is capable of producing the condition commonly known as sour beef.

MICROSCOPIC APPEARANCE OF SOUR BEEF

Several small pieces of artificially soured beef were prepared for sectioning by the paraffin method. Sections were made, mounted, and

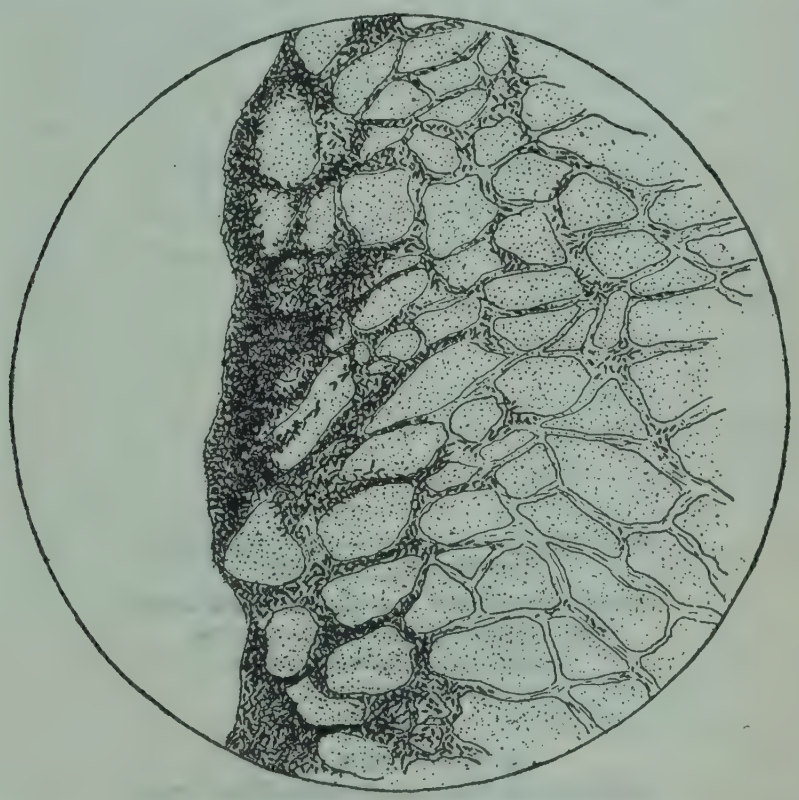

FIG. 2.—A cross section of beef muscle inoculated with organism 1510–B, stained by the Gram-Weigert method to show bacteria. Pen-and-ink drawing from microscope with aid of camera lucida.

stained by the Gram-Weigert method to show bacteria. Figure 2 is a reproduction of a pen-and-ink drawing made with camera lucida from one of the sections as seen under the microscope. The organisms are seen in large numbers on the exterior surface of the section, a fact which may be accounted for by the aerobic nature of the organisms. As they penetrated into the interior of the meat, growing between the muscle fibers, their multiplication was greatly inhibited by the anaerobic condition of the interior of the tissue, and yet a few persevering organisms may be seen to have penetrated well into the tissue.

CONCLUSIONS

The phenomenon known as the souring of beef is a bacterial one.

The organism responsible for the souring of beef is *Bacillus megatherium.*

Bacillus megatherium will sour beef under a wide range of temperatures, but not in the absence of oxygen.

In the souring of beef by *Bacillus megatherium* propionic acid is produced.

Bacillus megatherium is nonpathogenic for experimental animals (rabbits and guinea pigs) and does not produce an appreciable amount of toxin when propagated upon raw beef.

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A FUNGUS DISEASE SUPPRESSING EXPRESSION OF AWNS IN A WHEAT-SPELT HYBRID

By LLOYD E. THATCHER
Assistant Agronomist, Ohio Agricultural Experiment Station

During the winter of 1919, a number of F_2 hybrids between *Triticum vulgare* Vill. and *Triticum spelta* L. were grown in the greenhouse of the Department of Agronomy of the Ohio Agricultural Experiment Station. One bed was planted with 124 grains spaced about 8 inches apart each way. The seeds were first treated with the spores of *Tilletia foetans* (B. and C.) Trel., the object of the treatment being to isolate any segregates resistant to the smut that may have come out in the F_2 plants. That the treatment was successful is shown by the control.

An examination of the mature plants showed that they might be divided into three classes: Those of which all the grains of all the spikes were infected; those of which all the grains of part of the spikes were infected; and those of which all the grains of all the spikes were free from infection. The number of mature spikes varied from 1 to 6. Assuming a simple Mendelian ratio in which AA would represent the genotype of the all diseased, Aa the part diseased, and aa the disease-free, we would have the following classification:

Class.	Observed number.	Expected number.	Standard deviation.	Actual deviation.	Ratio.	
					Observed.	Expected.
All spikes diseased, AA.....	19	31	4.82	12	0.613	1
Not all spikes diseased, Aa.....	72	62	5.57	10	2.322	2
Total AA and Aa.....	91	93	4.82	2	2.935	3
All spikes disease-free, aa.....	33	31	4.82	2	1.065	1
Total AA, Aa, and aa....	124	124	.....	.....	4.000	4

It is observed that the class of the all diseased spikes is under the expectation and the class not all diseased is over the expectation. We may assume that the identification is at fault, AA sometimes giving

the same result as Aa. If the classes AA and Aa be taken as one, the deviation from expectancy is two, which is less than one-half the standard deviation.

An examination of the diseased plants revealed an interesting phenomenon as illustrated by Plate 135. Those plants which were awned segregates of the class in which part of the spikes were diseased showed normal development of the awns in the disease-free spikes and a suppression of awns in the diseased spikes. No spikes were found which contained both diseased and disease-free kernels.

PLATE 135

Spikes from three plants of F_2 wheat-spelt hybrid. The smutted spikes are on the left and the smut-free spikes on the right in each group.

EFFECT OF AMMONIUM SULPHATE UPON PLANTS IN NUTRIENT SOLUTIONS SUPPLIED WITH FERRIC PHOSPHATE AND FERROUS SULPHATE AS SOURCES OF IRON

By LINUS H. JONES and JOHN W. SHIVE
New Jersey Agricultural Experiment Station

INTRODUCTION

The experimental work reported in this paper grew out of a study of ammonium sulphate [$(NH_4)_2SO_4$] as a possible source of nitrogen for wheat and soybean plants in nutrient solutions. The status of the problem regarding the direct utilization of ammonium salts by agricultural plants is still in a very unsatisfactory condition. While experimental evidence favors the idea that ammonium salts are good sources of nitrogen for the higher plants, it also indicates that plants differ in their ability to utilize ammonium salts. Some plants appear to require the ammonium ion as well as the nitrate ion for growth at certain stages of their development. In some recent work Espino (*3*)[1] has shown that the rice plant during the early stages requires the ammonium ion for normal growth. From a review of the more important contributions to the literature on the mineral requirements of the rice plant, this author summarizes by saying:

> There is good reason to suppose that: (1) This plant requires the same chemical elements as do other higher plants. (2) The young plants are not suited to deriving their nitrogen from nitrates but thrive very well when ammonium sulphate (or possibly other ammonium salts, such as cholride, nitrate, etc.) is supplied. (3) Older plants are able to derive their nitrogen supply from nitrates, but may be able to thrive without the nitrate ion when the ammonium ion is supplied at a proper rate.

He calls attention to the fact, however, that—

> none of these points is at all well established in a quantative way.

The more recent work of Trelease and Paulino (*19*) and that of Trelease (*20*) is of interest in this connection. The results of their studies of the rice plant in soil cultures indicate that ammonium nitrogen is much more effective than nitrate nitrogen in increasing the yields of this plant. The yields obtained per unit of molecular nitrogen were highest when the nitrogen was applied to the cultures in the form of ammonium sulphate; they were lower when supplied as ammonium nitrate, and still lower when supplied in the form of calcium nitrate [$Ca(NO_3)_2$] or sodium nitrate ($NaNO_3$).

A review of the literature on the general subject of the utilization of ammonium as a source of nitrogen for the higher plants can not here be

[1] Reference is made by number (italic) to "Literature cited," p. 727-728.

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attempted. Mention should be made, however, of the important work of Hutchinson and Miller (*11*) in which they summarize a review of the more definite contributions to the literature on this broad subject. As a result of their own careful research on the direct assimilation of ammonium salts by higher plants, these authors conclude that agricultural plants can develop normally when supplied with nitrogen only in the form of ammonium salts, that some plants grow equally well with ammonium or nitrate as a source of nitrogen, and others, while capable of assimilating ammoniacal nitrogen, appear better able to utilize nitrates. They express the doubt, however—

whether ammonium salts can ever produce better final results than nitrates.

In some preliminary experiments with nutrient solutions of the Tottingham (*18*) type in comparison with these solutions in which the potassium nitrate (KNO_3) was replaced by ammonium sulphate in equivalent osmotic concentrations, it was found that the solutions containing ammonium sulphate were toxic to wheat and soybean plants when soluble ferrous sulphate ($FeSO_4$) in quantities of 1 mgm. of iron per liter of nutrient solution was added as a source of iron for the plants. When, however, equivalent amounts of iron in the form of the insoluble ferric phosphate ($FePO_4$) were added to the solutions containing ammonium sulphate, the toxic effect upon the plants entirely disappeared. The phenomena observed in connection with the use of the two forms of iron (soluble and insoluble) in the presence of the ammonium ion in complete nutrient solutions appeared to be of sufficient importance to warrant careful investigation. A comparative study was, therefore, made of the physiological effects produced upon wheat and soybean plants during the early stages of development by the two types of nutrient solutions just mentioned—the Tottingham solutions unmodified and these solutions in which ammonium sulphate was substituted for the potassium nitrate—with traces of a soluble ferrous compound and an insoluble ferric compound added as sources of iron for the plants. A study was also made of the influence of the plants upon the media in which they were grown. The results obtained with wheat will alone be presented in the present paper. Those for soybeans will appear in a later publication.

METHODS OF PROCEDURE

The experiments considered in the following pages each comprised two series of cultures. In the first of these two series, 20 representative solutions chosen from Tottingham's (*18*) complete series of 84 were used. The 20 solutions chosen are uniformly distributed throughout the series and will be designated by the culture numbers referring to the positions which they occupy in the series and on the 4-coordinate diagrammatic scheme employed by Tottingham. The second series was like the first in every respect, except that ammonium sulphate in equal osmotic con-

centrations was substituted for the potassium nitrate in the Tottingham solutions of the first series. With this substitution it is reasonable to suppose that any marked differences in the response of the plants toward the nutrient media in corresponding cultures of the two series could be attributed to the influence of the ammonium sulphate upon the plants either directly or indirectly, assuming the cultures to be subjected alike to all other experimental conditions. Two control solutions serving as standards for comparison were added to each series. These consisted of Tottingham's (*18*) best solution for wheat, number $T_3R_1C_4$, with a total osmotic concentration value of 2.5 atmospheres, and Shive's (*15*) best solution for wheat, number R_5C_2, with an osmotic concentration value of 1.75 atmospheres.

Baker's analyzed salts were used in the preparation of the solutions. Table I gives the culture numbers indicating the position of the cultures in the Tottingham series and the partial volume-molecular concentrations of the salts as they occurred in the solutions of the two series. The nutrient solutions of each series were made up from half-molecular stock solutions of the salts used to give to each solution a calculated total osmotic concentration value of 1 atmosphere. Freezing-point determinations showed that this concentration was closely approximated for each solution.

TABLE I.—*Description of solutions used*

Solution No.	Volume-molecular partial concentrations.[a]				
	Tottingham series (A).	Ammonium-sulphate series (B).			
	KNO_3.	KH_2PO_4.	$Ca(NO_3)_2$.	$MgSO_4$.	$(NH_4)_2SO_4$.
$T_1R_1C_1$	0.0020	0.00211	0.00146	0.01659	0.0014
C_3	.0020	.00211	.00438	.01185	.0014
C_5	.0020	.00211	.00730	.00711	.0014
C_7	.0020	.00211	.01022	.00237	.0014
R_3C_1	.0060	.00211	.00146	.01185	.0042
C_3	.0060	.00211	.00438	.00711	.0042
C_5	.0060	.00211	.00730	.00237	.0042
R_5C_1	.0100	.00211	.00146	.00711	.0070
C_3	.0100	.00211	.00438	.00237	.0070
R_7C_1	.0140	.00211	.00146	.00237	.0098
$T_3R_1C_1$	.0020	.00633	.00146	.01185	.0014
C_3	.0020	.00633	.00438	.00711	.0014
C_5	.0020	.00633	.00730	.00237	.0014
R_3C_1	.0060	.00633	.00146	.00711	.0042
C_3	.0060	.00633	.00438	.00237	.0042
R_5C_1	.0100	.00633	.00146	.00237	.0070
$T_5R_1C_1$	.0020	.01055	.00146	.00711	.0014
C_3	.0020	.01055	.00438	.00237	.0014
R_3C_1	.0060	.01055	.00146	.00237	.0042
$T_7R_1C_1$	.0020	.01477	.00146	.00237	.0014

[a] Osmotic concentration value of each solution 1 atmosphere.

Iron was supplied to all the cultures in equivalent amounts either in the form of the so-called insoluble ferric phosphate or the soluble ferrous sulphate. These two forms of iron were chosen in order to test the effect of a soluble and an insoluble form of iron upon growing plants in connection with the two types of nutrient solutions used, and still not introduce anions different from those provided by the four main salts present in the solutions in considerable quantities. The ferric phosphate was added to the solutions from a stock supply prepared as described in a previous publication (*12*), while the ferrous sulphate was added in the form of an aqueous solution freshly prepared each time just before being used in the culture solutions. This form of iron does not precipitate so rapidly nor so completely from the culture solutions here used as do other forms of soluble iron. By direct qualitative tests made from four to seven days after the ferrous sulphate was added to the culture solutions, the presence of dissolved iron could still be detected. As indicated by these qualitative tests, the capacity of iron citrate for maintaining its solubility in these solutions was equally as strong as that of ferrous sulphate. This is in accord with the view of Hoagland (*10*), who states that iron citrate and tartrate appear to be the most efficient sources of iron. The availability and efficiency of a given iron salt in relation to plant growth are, of course, determined largely by the concentration of the iron salt used and by the concentration, reaction, and composition of the nutrient solution in which it is used, as Gile and Carrero (*5*) have shown. Duggar (*2*) very successfully used what he calls "soluble ferric phosphate," which apparenlty consists of ferric phosphate with sodium citrate, as a single salt. He states that this salt combination possesses the advantage of solubility to a high degree and introduces no difficulties in the preparation of the solutions but may render their composition somewhat less definite.

The plants used as indicators consisted of spring wheat of the Marquis variety. The seeds were germinated on a germinating net like that used by Shive (*15*). All the seedlings used were carefully selected for uniformity of size and general appearance as to health and vigor and were transferred to the culture solutions when about 5 cm. tall. Three seedlings were comprised in each culture. The seedlings were mounted in the double-piece paraffined cork stoppers as devised by Tottingham (*18*). These were of the proper size to fit the quart fruit jars which were used as containers. To exclude light from the roots and to prevent heat absorption when the cultures were exposed to sunlight the containers were covered with cylindrical shells black within and light in color on the outside like those described by Shive (*15*).

The solutions were renewed regularly at intervals of 3½ to 4 days. At the time of each renewal of the solutions tests of the hydrogen-ion concentration of each of the old solutions was made by the colorimetric

method, using the standard buffer mixtures and indicators recommended by Clark and Lubs (*1*), and these concentrations were recorded in terms of P_H values.

The plants were grown in the culture solutions during a period of five weeks. At the end of the growth periods of the different experiments the dry weights of tops and roots of each culture were obtained separately by the usual method.

In order to obtain information regarding the nonsolution environment of the plants daily records were kept, in so far as this was possible, of the measurements characterizing the aerial conditions. These data are briefly summarized in Table II, and in this form they provide some fragmentary evidence as to the sort of aerial conditions which prevailed in the greenhouse during the several experimental periods.

TABLE II.—*Maximum and minimum temperatures, average daily water loss by evaporation from standard white and black spherical atmometers, and character of days for the different experiment periods*

Experiment No.	Experimental period.		Air temperature.		Average daily evaporation.			Radio evaporation.[a]			Number of days.		
	Beginning.	Ending.	Maximum.	Minimum.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Clear.	Partly cloudy.	Cloudy.
			°C.	°C.	Cc.	Cc.	Cc.	Cc.	Cc.	Cc.			
I.....	Jan. 19	Feb. 28	32.0	6.0	23.7	15.4	17.8	2.8	2.2	2.7	14	12	15
II....	Mar. 12	Apr. 17	35.5	7.0	23.3	12.4	18.9	4.4	1.1	2.5	19	7	11

[a] The values given for radic evaporation represent the average daily excess of water loss from standard black spherical atmometer over that from the white.

EXPERIMENTAL RESULTS

For the sake of convenience in presenting the data, the two series of cultures here considered in connection with each experiment will be designated the Tottingham series (A) and the ammonium-sulphate series (B). It has already been stated that the two series as used in the different experiments are alike in every respect except that ammonium sulphate in equivalent osmotic concentrations was substituted in the solutions of the latter for the potassium nitrate in the former.

In Table III are given the dry-weight yields of tops and of roots for the cultures of the two experiments considered. All yield values are expressed in terms of the value of the first culture ($T_1R_1C_1$) in the respective series considered as unity. The absolute yield value of this culture in grams is given just below the relative value 1.00 in each case. Since the high yields are always of greater interest than are the medium or low yields, the relative dry weights of tops and of roots from the six high-yielding cultures in each series are given in the table in bold-face type.

EXPERIMENT I. FERRIC PHOSPHATE AS THE SOURCE OF IRON IN THE SOLUTIONS OF SERIES A AND OF SERIES B

The importance of the proper amounts and forms of iron to be used with a given nutrient solution in order that the plants may be adequately supplied with this element for normal growth can scarcely be overestimated. As has been shown by Gile and Carrero (*6*) and pointed out also by Hoagland (*10*), the presence in culture solutions of sufficient available iron for the development of green plants depends largely upon the form and amount of the iron salt used, upon the reaction and concentration of the nutrient solution in which it is used, and upon the time of standing. It should be added also that the different species vary considerably in their requirements for this element and in their ability to obtain it from nutrient solutions, as has been clearly brought out in preliminary experiments in connection with this study.

TABLE III.—*Relative dry-weight yields of wheat tops and roots from Tottingham's series (A) and from the ammonium-sulphate series (B) with equivalent amounts of iron supplied to each culture in the form of ferric phosphate or ferrous sulphate* [a]

Culture No.	Experiment I, source of iron $FePO_4$.				Experiment II, source of iron $FeSO_4$.			
	Series A.		Series B.		Series A.		Series B.	
	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
$T_1R_1C_1$	1.00	1.00	1.00	1.00	1.00	1.00	**1.00**	**1.00**
	(.7352)	(.1975)	(1.1316)	(.2609)	(1.0389)	(.2452)	(.9016)	(.1724)
C_3	1.35	**1.67**	**1.95**	**2.41**	**3.17**	**2.98**	.95	**1.01**
C_5	1.32	1.02	**2.05**	**2.30**	2.61	2.65	**.96**	**1.04**
C_7	.72	.91	1.10	1.53	1.39	1.79	.74	.90
R_3C_1	.92	1.11	1.26	1.11	2.04	1.35	.74	.84
C_3	.94	1.27	1.50	1.42	**3.26**	**2.93**	.81	.87
C_5	.79	.96	**1.72**	**1.61**	2.47	**3.38**	.57	.58
R_5C_1	.82	.86	.98	.92	2.55	2.12	.65	.65
C_3	.65	.96	1.12	1.23	2.83	**2.89**	.61	.58
R_7C_1	.78	1.22	.96	1.46	2.42	1.63	.69	.70
$T_3R_1C_1$	**1.44**	1.47	1.40	**1.80**	1.85	1.47	**1.16**	**1.16**
C_3	**1.40**	**1.87**	**1.68**	**2.22**	2.97	**3.26**	**1.09**	**1.10**
C_5	1.20	**1.77**	1.18	1.42	**2.99**	**3.02**	.84	.54
R_3C_1	**1.48**	1.42	**1.50**	**1.72**	**3.15**	1.63	**1.01**	.96
C_3	1.20	1.16	1.49	1.57	2.17	2.20	.76	.76
R_5C_1	1.18	1.37	1.15	1.27	**3.24**	2.77	.79	.74
$T_5R_1C_1$	**1.36**	**1.62**	**1.53**	1.15	2.37	1.92	**1.46**	**1.40**
C_3	1.31	**1.72**	1.13	1.04	1.81	2.00	.94	.97
R_3C_1	**1.61**	1.57	1.42	1.42	**2.99**	2.00	.79	.74
$T_7R_1C_1$	**1.69**	**1.87**	1.13	1.34	2.96	2.85	.94	.95
Shive's R_5C_2	1.73	2.09	.....	.....	2.39	1.84	.....	.....
Tottingham's $T_3R_1C_4$	1.65	2.33	.....	.....	2.49	3.30	.....	.....

[a] Data from the six high-yielding cultures are given in bold-face type.

Iron salts are, of course, more soluble in solutions of higher hydrogen-ion concentration than they are in solutions with lower concentrations of the hydrogen ions; but as Hoagland (*10*) has pointed out, certain forms of iron may be completely precipitated in a relatively short time when the total phosphate content of the solution is high. It appears, however, that the insoluble ferric phosphate used in the nutrient solutions here considered may be made available to the plants under certain conditions, as will be brought out by a study of the data presented in connection with these experiments.

In the first experiment each culture solution of the two series used was supplied with 0.83 mgm. of iron per liter of nutrient solution in the form of an aqueous suspension of ferric phosphate. This amount of iron was added to each culture jar at the time the solutions were renewed.

GENERAL APPEARANCE OF THE PLANTS

At the beginning of the third week of the growth period the plants in some of the cultures of the Tottingham series began to show the yellow appearance which is characteristic of plants suffering from lack of iron. This chlorotic condition later became general throughout the series. The yellowing of the leaves was usually more pronounced in the cultures having salt proportions which were unfavorable for good growth and in which the plants were smaller as a result of this unbalanced condition of the solutions. In general, however, there was good agreement in the appearance of the three plants in any one culture. That the chlorotic condition of the plants in this series was caused by the lack of available iron was made clear by the use of supplementary cultures to which iron in the form of ferrous sulphate was added in amounts equivalent to those contained in the ferric phosphate supplied to each culture. The chlorotic plants in these cultures invariably regained the normal green color of healthy plants in the course of a few days after the soluble iron was added. At the end of the growth period the plants in most of the cultures of the Tottingham series were very chlorotic and not nearly so large as were the plants in the corresponding cultures of the ammonium-sulphate series, these differences in size being paralleled by similar differences in the dry-weight yields, as an inspection of the data in Table III will show. The plants in the cultures of the ammonium-sulphate series, on the other hand, were very green; and in so far as could be judged from their general appearance they were vigorous and healthy, with the exception of the plants in several cultures which were not quite so green in color but otherwise appeared to be in good condition. The slight yellowness in the plants of these few cultures was of a different nature from that which occurred in the plants of the Tottingham series. There were, of course, large differences in the size of the plants of the different cultures resulting from the differences in salt proportions throughout the series. There was, however, no evidence of the lack of chlorophyll in the leaves, such as characterized the plants of the Tottingham series.

INFLUENCE OF THE GROWING PLANTS ON THE HYDROGEN-ION CONCENTRATION OF THE SOLUTIONS

It is, of course, well known that plants tend to change the reaction of culture solutions in which they are grown. A large number of supplementary tests made in connection with this work have brought out the fact that the rate of this change in a culture solution is dependent upon a number of different factors, some of the more important of which are

the total concentration of the solution, the relative proportions of the salt constituents, the total quantity of solution per plant, the age of the plants, and the condition of their aerial environment. From observations made during the present study the last-named factor appears to have considerable influence in determining the rate of change of the hydrogen-ion concentration of the culture solutions here used.

As previously stated, the hydrogen-ion concentrations of the culture solutions were determined at the end of the growth intervals between each two successive solution renewals throughout the entire growth period. In Table IV are presented the summarized data of these determinations in terms of P_H values. Thus, in the table are given the initial P_H values of the culture solutions, the highest and the lowest values obtained, and the average of all the values for each solution of the two series. Four days previous to harvesting the different cultures were compared with respect to yellowness of the leaves. The comparisons were made and values were obtained by means of the relative score method described by Free (*4*) for recording unmeasured plant characters. To facilitate comparisons, the results of this score are given in Table IV in connection with the hydrogen-ion exponents, the lowest score value for any culture of the two series being considered as unity.

TABLE IV.—*P_H values and score for yellowness of leaves of the culture solutions of the Tottingham series (A) and the ammonium-sulphate series (B) supplied with iron in the form of ferric phosphate*

Culture No.	Tottingham series (A).					Ammonium-sulphate series (B).				
	Score for yellowness.	P_H values.				Score for yellowness.	P_H values.			
		Initial.	Highest.	Lowest.	Average.		Initial.	Highest.	Lowest.	Average.
$T_1R_1C_1$	7.0	4.8	6.0	5.0	5.47	0.0	4.8	5.1	4.4	4.68
C_3	7.6	4.9	6.2	5.2	5.67	.0	4.8	5.4	4.2	4.75
C_5	7.7	4.8	6.2	5.1	5.70	.0	4.9	5.9	4.2	4.85
C_7	7.7	4.8	5.8	5.0	5.46	2.0	4.9	6.0	4.4	5.10
R_3C_1	7.7	4.8	6.1	5.1	5.55	.0	4.9	5.8	4.2	4.76
C_3	1.3	4.8	6.0	5.2	5.61	.0	4.9	6.0	4.1	4.75
C_5	7.7	4.9	6.0	5.2	5.55	1.0	4.9	5.0	4.1	4.39
R_5C_1	7.8	4.9	5.9	5.0	5.57	1.9	4.9	5.6	4.1	4.62
C_3	7.7	4.8	6.0	5.2	5.60	1.0	4.9	5.7	4.1	4.53
R_7C_1	6.6	4.9	6.0	5.5	5.71	2.0	4.9	5.9	4.0	4.59
$T_3R_1C_1$	4.2	4.6	5.9	4.8	5.33	.0	4.6	5.6	4.2	4.70
C_3	4.2	4.7	5.9	4.8	5.39	.0	4.6	5.2	4.4	4.69
C_5	4.2	4.8	5.9	4.9	5.33	.0	4.8	5.8	4.6	4.89
R_3C_1	4.2	4.8	5.9	5.0	5.39	.0	4.7	5.2	4.1	4.50
C_3	4.2	4.8	6.0	4.8	5.40	.0	4.8	4.9	4.1	4.47
R_5C_1	7.6	4.8	5.9	5.1	5.49	.0	4.8	5.7	4.1	4.72
$T_5R_1C_1$	4.3	4.6	5.8	4.9	5.24	.0	4.6	5.7	4.4	4.84
C_3	4.4	4.7	5.9	4.7	5.32	.0	4.6	5.6	4.2	4.72
R_3C_1	4.3	4.6	5.9	4.8	5.36	.0	4.6	5.5	4.1	4.66
$T_7R_1C_1$	4.4	4.6	5.9	4.8	5.32	1.8	4.6	5.4	4.4	4.70
Shive's—										
R_5C_2	1.8	4.6	5.6	4.6	4.96					
Tottingham's—										
$T_3R_1C_4$	4.2	4.6	5.4	4.6	4.94					

The data of Table IV show that the hydrogen-ion concentrations of the culture solutions of the Tottingham series were always considerably

decreased during any growth interval. The culture solutions of the ammonium-sulphate series, on the other hand, usually increased somewhat in hydrogen-ion concentrations. The average P_H values of the culture solutions of this series do not vary greatly from the initial values. For most of the solutions these average values were slightly lower and a few were slightly higher than were the initial P_H values of the solutions.

To bring out more clearly the magnitude and the direction of the change in reaction of the culture solutions during the different intervals throughout the growth period, detailed hydrogen-ion concentration data for the culture solutions producing the highest, medium, and lowest yields in each of the series are given in Table V. From the data of this table and those of Table IV it can be definitely stated that the effect of the growing plants was always to decrease the hydrogen-ion concentration of the culture solutions of the Tottingham series and to increase the concentration of the hydrogen ions of the solutions of the ammonium-sulphate series, or, at least, to maintain the initial concentrations of these ions during the first four weeks of the growth period.

TABLE V.—P_H *values of the culture solutions of the Tottingham series (A) and the ammonium-sulphate series (B) producing highest, medium, and lowest yields with iron supplied in the form of ferric phosphate* [a]

Dates of solution renewals and growth intervals.	Tottingham series (A) producing—			Ammonium-sulphate series (B) producing—		
	Highest yield ($T_7R_1C_1$).	Medium yield ($T_3R_1C_5$).	Lowest yield ($T_1R_5C_3$).	Highest yield ($T_1R_1C_5$).	Medium yield ($T_3R_3C_3$).	Lowest yield ($T_1R_7C_1$).
1919.						
Aug. 22	4.6	4.8	4.8	4.9	4.6	4.9
26	4.8	4.9	5.2	4.9	4.8	4.5
29	4.9	5.0	5.4	5.0	4.8	4.8
Sept. 2	5.0	5.1	5.4	4.8	4.6	4.4
5	5.1	5.1	5.3	4.6	4.6	4.4
9	5.4	5.4	5.5	4.2	4.7	4.1
12	5.3	5.3	5.5	4.6	4.6	4.2
16	5.7	5.6	5.9	4.4	4.6	4.1
19	5.5	5.6	5.8	4.2	4.4	4.0
23	5.9	5.9	6.0	5.9	5.2	5.9
27	5.6	5.4	6.0	5.9	4.6	5.5
Average	5.32	5.33	5.60	4.85	4.69	4.59

[a] The first number in each column represents the initial P_H value of the solution.

The exact manner in which the hydrogen-ion concentrations of the nutrient solutions are altered by the growing plants has not yet been clearly demonstrated. Hoagland (*8*) has suggested that the change in reaction must be the result of the secretion of neutralizing substances by the plants, of chemical reaction with the material of the roots, or of the selective absorption of specific ions. As a result of extensive studies of absorption of ions by plants, Pantanelli (*13*) ascribes the change in reaction of the nutrient media mainly to the differential absorption of the ions by the living plant roots.

It appears that the type of the culture solution as determined by the nature of ion constituents is in a large measure responsible for the direction of the change in reaction produced by the growing plants. Hoagland (*10*) in a recent publication has emphasized the point that a nutrient solution is an exceedingly complex system and that it does not appear possible at the present time to determine quantitatively the ions and undissociated salts which it may contain during or after the growth of plants in it, much less does it appear possible to determine the exact relationship existing between the different components of such a complex system. In an earlier publication (*8*) the last-named author came to the conclusion that plants may regulate the reaction of the nutrient medium in such a way that excessive hydrogen or hydroxyl ions can not occur. In later work (*9*) he found that barley plants grown for seven weeks in a favorable nutrient solution when transferred to single salt solutions of various salts did not bring about an unfavorable condition of acidity or alkalinity. In this work, however, he noted that ammonium-chlorid solutions retained an acid reaction and the hydrogen-ion concentration was slightly increased after contact with the plants. Toole and Totting-ham (*17*), working with Knop's solution, call attention to the fact that growing barley seedlings always had a marked neutralizing effect upon the medium. It is of interest here to note that plants grown in the Shive's (*15*) 3-salt solution always show a marked tendency to decrease the hydrogen-ion concentrations, but when ammonium sulphate, even in relatively small amounts, is superimposed upon this solution the direction of the change in reaction is reversed, just as it is when this salt is substituted for potassium nitrate in the Tottingham solutions. This is true of plants during at least the first four weeks of growth.

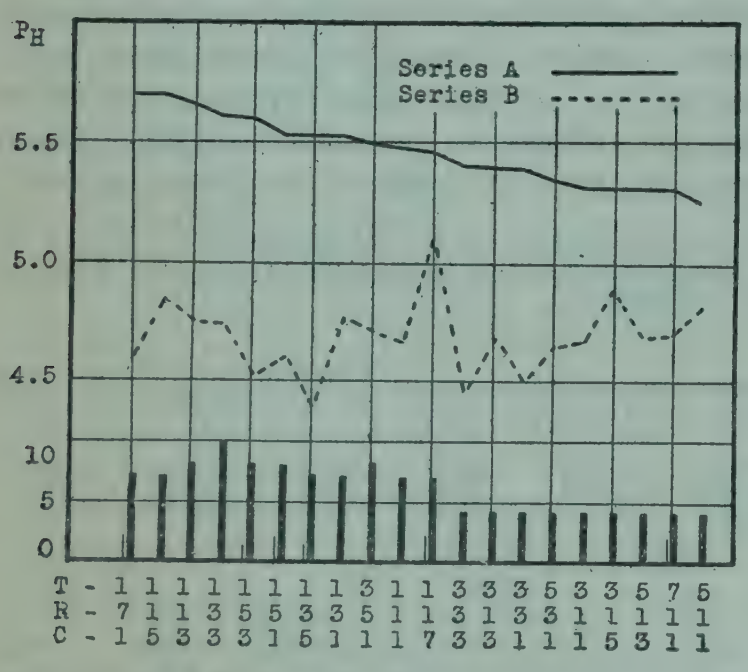

FIG. 1.—Graphs of PH values of culture solutions after contact with plant roots during growth interval between solution renewals; averages of tests during growth period of experiment I. Also diagram of score values for yellowness. Broad and narrow vertical lines represent score for plants of Tottingham series and ammonium-sulphate series, respectively.

Inspection of the data in Table IV brings out the fact that the initial P_H values of the corresponding solutions of the two series are approximately the same. The averages of the P_H values determined at the end of the various growth intervals are, however, always much higher for the

culture solutions of the Tottingham series than they are for the corresponding ones of the ammonium-sulphate series. This is brought out more clearly by the graphs of figure 1, which represent the average P_H values plotted in the descending order of their magnitudes in the Tottingham series. The relative values of the score for yellowness for the cultures of the Tottingham series and of the ammonium-sulphate series are represented graphically by broad and narrow vertical lines, respectively, just below the graphs representing the average P_H values.

Comparing now the score for yellowness of leaves with the average P_H values obtained at the end of the growth intervals, it will be observed that the higher P_H values throughout the Tottingham series correspond in a general way to high relative score values for yellowness in this series. The P_H values throughout the series decrease slightly with increase in the phosphate content of the solutions, and these slight differences are generally paralleled by somewhat more pronounced differences in the relative values of the score for yellowness.

In the cultures of the ammonium-sulphate series the plants were entirely free from any chlorotic condition such as characterized the plants in the Tottingham series the culture solutions of which had much higher average P_H values. This is also apparent from the color score, which, however, shows a slight degree of yellowness for the plants of several cultures only of the ammonium-sulphate series to which reference has previously been made.

Attention has already been called to the fact that the chlorotic condition of the plants in the Tottingham series was due to an insufficient supply of available iron, since the addition of soluble iron to the solutions of such cultures enabled the plants to regain their normal green color and to overcome completely the chlorotic condition in the course of a few days. There was no evidence, however, of the lack of available iron in the culture solutions of the ammonium-sulphate series. Thus, by the substitution of ammonium sulphate for potassium nitrate in the Tottingham solutions, the ferric phosphate here used as a source of iron for the plants was rendered available perhaps by the maintenance of a higher average concentration of the hydrogen ions during the growth of the plants in the solutions, since the solubility of iron is, of course, greater in solutions of higher hydrogen ion concentrations.

DRY-WEIGHT YIELDS OF TOPS AND ROOTS

The relative dry-weight yields of tops and of roots from the cultures of the two series here considered are given in Table III. The absolute dry-weight yield values corresponding to these relative data have been plotted to form the graphs of figures 2 and 3, in which the ordinates represent dry weights in grams and the abscissas represent the different cultures. The values are always plotted according to the descending order of their magnitudes in the Tottingham series.

The graphs of figure 2 show at once that the yields of tops from the cultures of the ammonium-sulphate series are much superior to the yields from the corresponding cultures of the Tottingham series. In general this is true also with respect to the yields of roots from the two series as is indicated by the graphs of figure 3. However, the root yields from three cultures of the ammonium-sulphate series are actually lower than are the yields from the corresponding cultures of the Tottingham series. The superiority of the yields from the ammonium-sulphate series is clearly not due to a greater efficiency of the four main salt constituents or combinations of the solutions of the series but is the direct result of the difference in the availability of the iron in the form here used in the solutions of the two series during the first five weeks of growth.

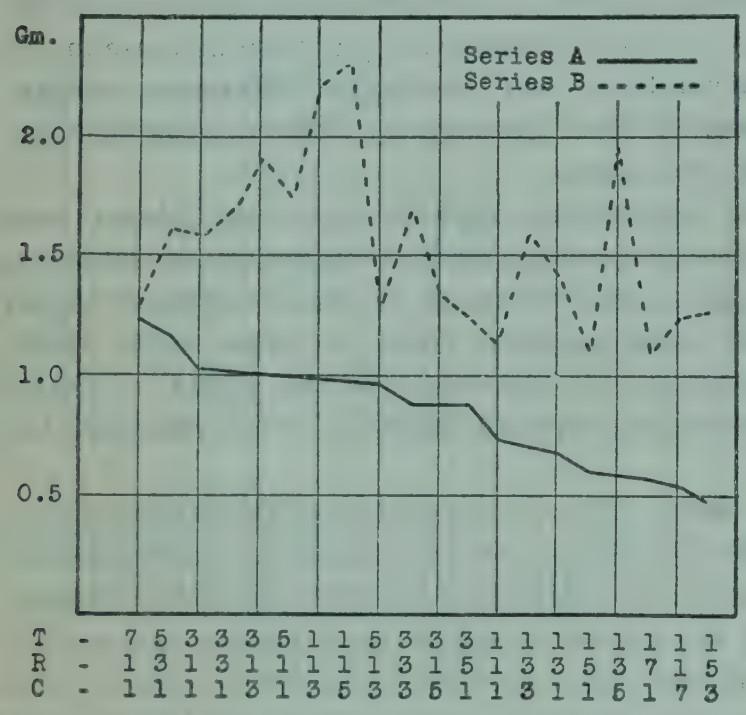

FIG. 2.—Graphs of actual yield vaules of wheat tops for the Tottingham series and the ammonium-sulphate series of Experiment I.

In the Tottingham series the growth of the plants was greatly retarded after the third week of growth by a deficiency in the supply of available iron, while no such deficiency of iron was at any time apparent in the culture solutions of the ammonium-sulphate series. The nature of the nutrient solutions with respect to their salt constituents and hydrogen-ion concentration here appears to determine the availability and the efficiency of the ferric phosphate as a source of iron for the plants. In connection with this study the work of Wolkoff (*21*) is of interest. In a sand culture procedure with soybeans this author used the same series of nutrient solutions employed in the present study, but the solutions had an osmotic concentration value of 2.5 atmospheres and iron was supplied in the form of iron rust. A marked superiority of the yields from the culture containing ammonium sulphate over those from the Tottingham solutions was shown, but the author states that the most pronounced differences in the plants from the two series of cultures were those of color, the plants from the cultures containing ammonium sulphate always being much greener than those from the Tottingham series.

EXPERIMENT II. FERROUS SULPHATE AS THE SOURCE OF IRON IN THE SOLUTIONS OF SERIES A AND SERIES B

The solution cultures used in the two series of this experiment were the same as those of the preceding experiment except that soluble ferrous sulphate was used as the source of iron for the plants instead of the insoluble ferric phosphate used in experiment I. As before 0.83 mgm. of iron per liter of nutrient solution was supplied to each culture jar at the time when the solutions were renewed. The ferrous sulphate was added in the form of an aqueous solution which was always freshly prepared just before being used. The treatment of the plants before and during the growth period, the renewal of the solutions, and the general culture methods were carried out in the same manner as were those in the preceding experiment.

GENERAL APPEARANCE OF THE PLANTS

During the first two weeks of the growth period there was no marked difference in the general appearance of the plants in the two series. The plants in the Tottingham series as a whole presented perhaps a slightly better appearance and showed a somewhat sturdier growth than did the plants in the ammonium-sulphate series. After 20 days the plants in some of the cultures of the ammonium-sulphate series showed a tendency to become weakened; the leaves drooped, presenting angles instead of curves at the weakened points, and the growth rates were greatly retarded. Later this condition became general throughout the series and increased in intensity as the plants grew older, giving them a very dull, unhealthy appearance. In a few cultures of this series another symptom of physiological disturbance appeared in the form of narrow white stripes in the leaves. This condition was quite unlike the chlorotic appearance of the leaves of plants suffering from lack of iron.

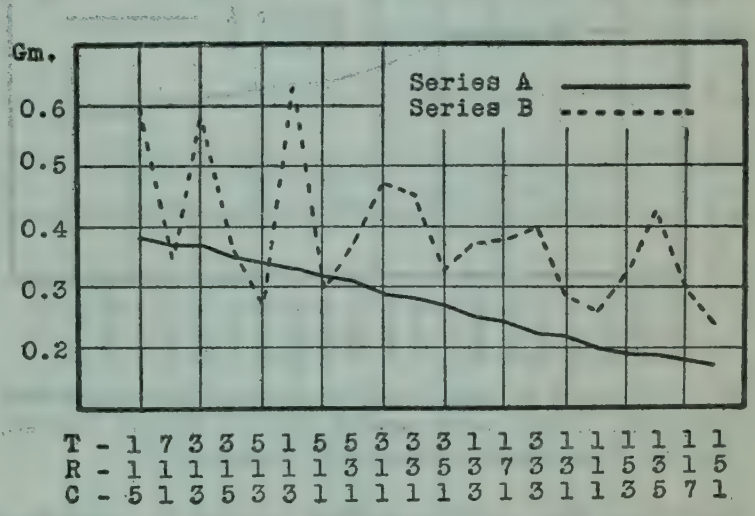

FIG. 3.—Graphs of actual yield values of wheat roots for the Tottingham series and the ammonium-sulphate series of experiment I.

The plants of the ammonium-sulphate series here presented a sharp contrast to those of the Tottingham series in which no toxic symptoms occurred at any time during the growth period. The plants in the latter series were at all times dark green in color and appeared vigorous and healthy, making rapid growth. There were, of course, large differences

in the size of the plants of the different cultures in this series as a result of variations in the salt proportions of the culture solutions, as the dry weights in Table III will show. Thus, by the use of the soluble ferrous sulphate in small quantities, instead of the insoluble ferric phosphate used in the solutions of the preceding experiment, the general health and vigor of the plants in the Tottingham series was very greatly improved, while the opposite effect was experienced by the plants in the cultures of the ammonium-sulphate series.

INFLUENCE OF THE PLANTS UPON THE HYDROGEN-ION CONCENTRATION OF THE MEDIA

The summarized data of the hydrogen-ion concentration determinations of this experiment are given in Table VI in terms of P_H values. The data of this table correspond in every respect to the similar data of Table IV. From the data of Table VI it is again evident that the growing plants in the Tottingham series showed a marked tendency to change the reaction of the solutions toward neutrality, while the direction of this change in reaction was reversed by the growing plants in the solutions of the ammonium-sulphate series. In the latter series the average of the P_H values obtained at the end of the several growth intervals was lower for each culture solution throughout the entire series than was the corresponding initial P_H value, although the increase in the hydrogen-ion concentration was not very great in any case.

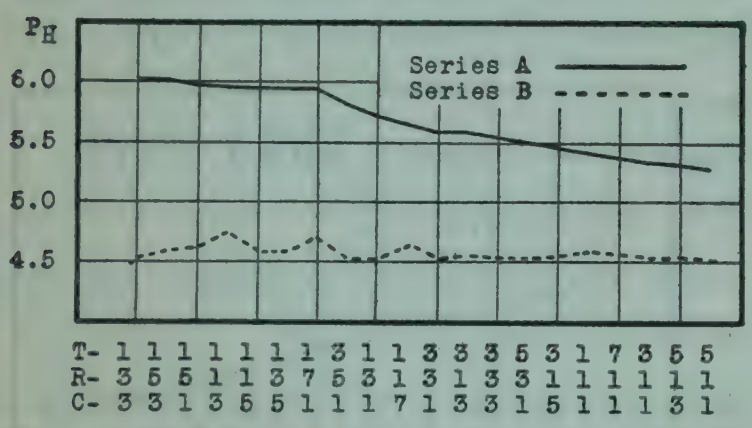

FIG. 4.—Graphs of P_H values of culture solutions after contact with plant roots during growth interval between solution renewals; averages of tests during growth period of experiment II.

The averages of the P_H values determined at the end of the various growth intervals are always much higher for the cultures of the Tottingham series than they are for the corresponding cultures of the ammonium-sulphate series. This is clearly brought out by the graphs of figure 4. A direct comparison of these graphs with the corresponding ones of the preceding experiment (fig. 1) shows that the two sets of graphs are generally in good agreement. This indicates that the influence of the growing plants upon the reaction of the culture solutions here used was practically the same when the iron was supplied in the form of the insoluble ferric phosphate or in the form of the soluble ferrous sulphate.

The detailed hydrogen-ion concentration data for the culture solutions producing highest, medium, and lowest yield given in Table VII show in a general way the direction and the magnitude of the change in

reaction in these solutions produced by the plants during the various intervals throughout the growth period. The data of this table, which agree in a general way with the corresponding data of Table V, are fairly representative of similar data for all the cultures of the respective series in this experiment. They further emphasize the point already brought out, that the different forms of iron here used have little if any effect upon the plants with respect to their influence upon the hydrogen-ion concentration of the nutrient media, except in so far as differences in size and vigor of the plants or differences in the extent of the absorbing surfaces of the root systems may produce somewhat corresponding differences in the magnitudes of the changes in reaction. On the other hand, the different forms of iron used in either of the two types of solutions here used produced markedly different effects upon the general appearance, health, and vigor of the plants and upon the growth rates, as will be more fully brought out by a further consideration of the dry-weight yields of tops and roots.

TABLE VI.—P_H *values of the culture solutions of the Tottingham series (A) and the ammonium-sulphate series (B) supplied with iron in the form of ferrous sulphate*

Culture No.	Tottingham series (A).				Ammonium-sulphate series (B).			
	Initial.	Highest.	Lowest.	Average.	Initial.	Highest.	Lowest.	Average.
$T_1R_1C_1$	4.8	6.0	4.8	5.42	4.8	4.7	4.4	4.59
C_3	4.8	6.6	5.0	5.97	4.8	5.0	4.5	4.74
C_5	4.8	6.6	5.0	5.96	4.8	4.7	4.4	4.60
C_7	4.8	6.3	4.9	5.65	4.9	4.8	4.4	4.63
R_3C_1	4.8	6.3	4.9	5.72	4.8	4.9	4.4	4.53
C_3	4.8	6.6	5.0	6.03	4.9	4.6	4.4	4.53
C_5	4.8	6.6	5.0	5.96	4.8	4.7	4.4	4.60
R_5C_1	4.8	6.6	4.9	5.98	4.9	4.7	4.4	4.61
C_3	4.8	6.6	4.9	6.03	4.9	4.8	4.4	4.59
R_7C_1	4.8	6.6	5.0	5.96	4.9	5.1	4.4	4.71
$T_3R_1C_1$	4.7	5.9	4.6	5.33	4.7	4.7	4.4	4.55
C_3	4.6	6.3	4.6	5.59	4.7	4.7	4.4	4.56
C_5	4.7	6.0	4.7	5.48	4.7	4.7	4.3	4.55
R_3C_1	4.7	6.3	4.7	5.60	4.7	4.7	4.4	4.53
C_3	4.7	6.3	4.6	5.54	4.7	4.6	4.4	4.54
R_5C_1	4.7	6.5	4.9	5.81	4.7	4.6	4.4	4.54
$T_5R_1C_1$	4.6	5.9	4.5	5.28	4.7	4.6	4.4	4.54
C_3	4.6	5.8	4.6	5.32	4.6	4.7	4.4	4.56
R_3C_1	4.7	6.2	4.6	5.50	4.6	4.6	4.4	4.54
$T_7R_1C_1$	4.6	5.9	4.5	5.37	4.6	4.7	4.4	4.56
Shive's R_5C_2	4.6	5.6	4.6	5.09	.....	.....	.....	.....
Tottingham's $T_3R_1C_4$	4.6	5.5	4.6	5.06	.....	.....	.....	.....

TABLE VIII.—P_H *values of the culture solutions of the Tottingham series (A) and the ammonium-sulphate series (B) producing highest, medium, and lowest yields with iron supplied in the form of ferrous sulphate*[a]

Dates of solution renewals and growth intervals.	Tottingham series (A) producing—			Ammonium-sulphate series (B) producing—		
	Highest yield ($T_1R_3C_3$).	Medium yield ($T_3R_3C_3$).	Lowest yield ($T_1R_1C_1$).	Highest yield ($T_5R_1C_1$).	Medium yield ($T_3R_3C_1$).	Lowest yield ($T_1R_3C_5$).
1920.						
Mar. 12	4.8	4.7	4.8	4.7	4.7	4.8
16	5.0	4.6	4.8	4.4	4.4	4.6
19	5.3	4.9	5.1	4.6	4.4	4.5
23	5.6	5.2	5.2	4.5	4.5	4.5
26	5.9	5.3	5.3	4.4	4.4	4.4
30	6.3	5.6	5.5	4.6	4.5	4.7
Apr. 2	6.3	5.7	5.4	4.6	4.7	4.6
6	6.3	5.6	5.3	4.6	4.6	4.7
9	6.5	5.9	5.7	4.6	4.6	4.6
13	6.6	6.3	5.9	4.6	4.6	4.7
17	6.5	6.3	6.0	4.6	4.6	4.7
Average	6.03	5.54	5.42	4.54	4.53	4.60

[a] The first number in each column represents the initial P_H value of the solution.

DRY WEIGHTS OF TOPS AND ROOTS

The relative yield values for the cultures of the two series of this experiment are given in Table III in connection with the relative dry-weight yields from the cultures of the preceding experiment. The absolute dry weights of tops and roots were plotted to form the graphs of figures 5 and 6, respectively. These graphs were prepared in the same manner as those representing the dry-weight yields in figures 2 and 3. From the graphs of figures 5 and 6 it will be observed that the yields of both tops and roots from the cultures of the Tottingham series were always much superior to those from the corresponding cultures of the ammonium-sulphate series, in which only a single culture gave a dry-weight yield of tops which was higher than the lowest yield of tops in the Tottingham series. In the ammonium-sulphate series all the root yields were lower than the lowest yield in the Tottingham series.

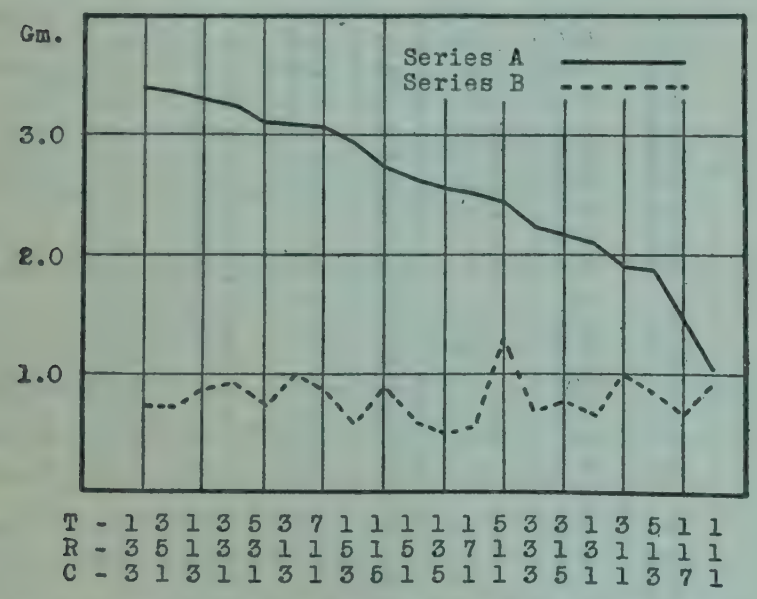

FIG. 5.—Graphs of actual yield values of wheat tops for the Tottingham series and the ammonium-sulphate series of experiment II.

Comparing now the graphs of figure 5 with those representing the dry-weight yields of tops from the two series of the preceding experiment (fig. 2), it will be observed that the relative positions of the graphs representing corresponding series of the two experiments are completely reversed. Thus, in experiment I (fig. 2) the graphs representing top yields from the cultures of the Tottingham series, throughout its entire length, lies much below the graph representing these yields from the ammonium-sulphate series, while the corresponding graphs in experiment II (fig. 5) occupy exactly opposite positions. A similar comparison of the two sets of graphs representing the dry-weight yields of roots from the two series in each experiment (fig. 3, 6) shows that these graphs have the same sort of arrangement as the graphs representing the dry weight of tops. It should be noted, however, that the differences between the yield values of tops, and especially of roots, from corresponding cultures of the two series, as indicated by the pairs of graphs representing the yields in question, are always much less pronounced in experiment I than they are in experiment II.

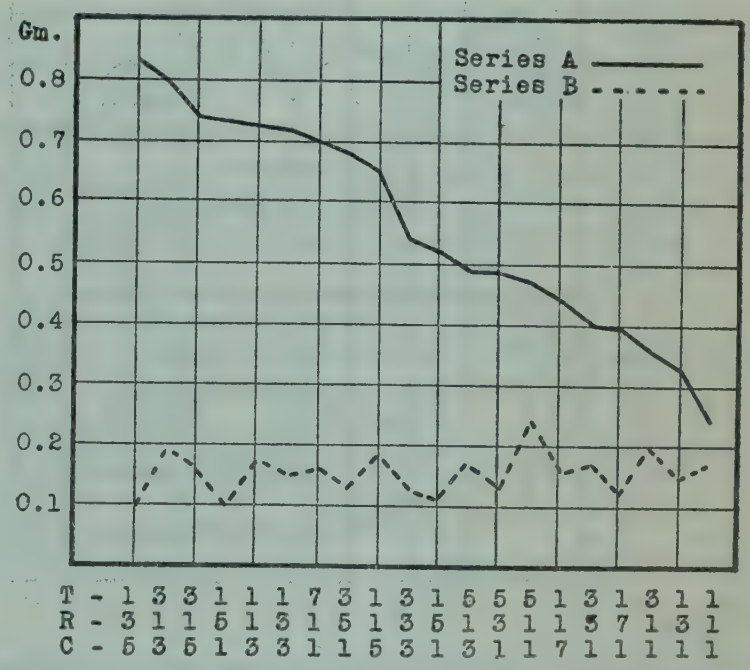

FIG. 6.—Graphs of actual yield values of wheat roots for the Tottingham series and the ammonium-sulphate series of experiment II.

The small amounts of iron used here with each culture (0.83 mgm. per liter of solution) appeared to be sufficient to prevent chlorotic effects in the plants of the Tottingham series and to provide them with the necessary supply of this element for normal growth; and, as already stated, the plants of this series were in excellent condition during the entire growth period and produced very high yields. In the solutions of the ammonium-sulphate series, on the other hand, the soluble iron in the small quantities used with each culture was very toxic to the plants, which were greatly retarded in their growth rates and produced very low yields. That this condition of the plants was directly related to the form of iron supplied is clear from the fact that in the preceding experiment these same culture solutions provided with iron, in equivalent amounts, in the form of the insoluble ferric phosphate, produced healthy, vigorous plants and high yields.

In order to test the effect of varying amounts of iron in the form of the soluble ferrous sulphate upon the growth of wheat plants in the two types of culture solutions used here, a special experiment was carried

out. This comprised two series of 10 cultures each. Throughout each series the same solutions were used for all the cultures, these solutions differing only in the amounts of iron contained. For one of these series Tottingham's solution number $T_3R_1C_5$ was chosen, and for the other this solution was modified by substituting ammonium sulphate for the potassium nitrate, as previously explained, in equivalent osmotic concentration (solution number $T_3R_1C_5$ of the ammonium-sulphate series in experiments I and II). These two series will be designated series C and series D. Iron was supplied to the solutions of each series in amounts varying from 0.01 mgm. to 5.0 mgm. The culture methods pursued with these two supplementary series were precisely the same as those described and used in carrying out the main experiments of this study. The cultures were conducted during a growth period of 35 days.

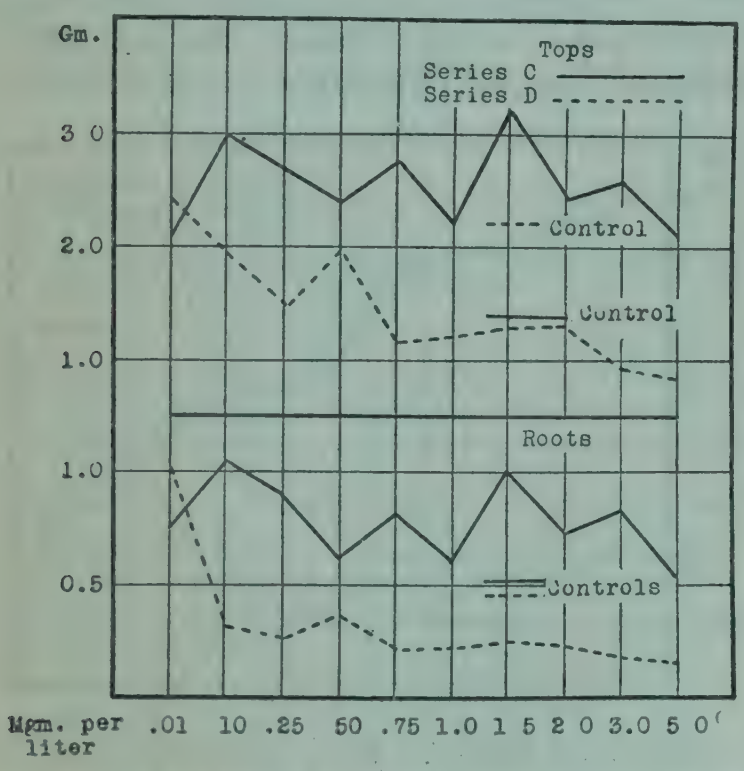

FIG. 7.—Graphs of actual yield values of wheat tops and roots for culture solutions of two types (solution $T_3R_1C_5$ of the Tottingham series and solution $T_3R_1C_5$ of the ammonium-sulphate series) supplied with varying amounts of iron in the form of ferrous sulphate. Series C and D of experiment II.

During the third week of the growth period evidences of toxicity began to appear in the plants growing in the solutions containing ammonium sulphate. The plants assumed an unhealthy, slightly yellowish, dull color, which was entirely different from the characteristic chlorotic appearance of plants suffering from the insufficient supply of iron. A weakened condition of the plants was evident from the manner in which the leaves drooped and the growth rates were retarded. The plants in the solutions containing the highest amounts of iron were the first to be affected, and the toxicity increased in severity as the plants grew older and spread to include all of the cultures except the one containing the smallest amount of iron (0.01 mgm. per liter of solution). The plants in this culture were at all times green and healthy, grew rapidly, and produced relatively high yields. The plants in the Tottingham solutions were green and healthy throughout the growth period, with the exception of those in the two solutions containing 0.01 mgm. and 0.10 mgm. of iron per liter of solution. The plants in the first of these two solutions were chlorotic, while those in

the second were only slightly chlorotic as the result of an insufficient supply of available iron.

At the end of the growth period of 35 days the dry weights of tops and roots were obtained in the usual way. The yield for the cultures of the two series, together with the average hydrogen-ion exponents of the solutions obtained at the end of the various growth intervals, are given in Table VIII. The yield values of tops and roots as given in this table are represented graphically in figure 7, the upper set of graphs representing the yields of tops and the lower set the root yields. The dry-weight values as ordinates are here plotted against the amounts of iron in milligrams per liter of solution as abscissas.

TABLE VIII.—*Hydrogen-ion concentrations and dry-weight yields of wheat grown in two types of nutrient solutions supplied with varying quantities of iron in the form of ferrous sulphate*

Quantities of iron (in milligrams per liter of solution).	Tottingham's solution ($T_3R_1C_5$), series C.			Tottingham's solution ($T_3R_1C_5$) modified, [$(NH_4)_2SO_4$ substituted for KNO_3], series D.		
	Dry weight of tops	Dry weight of roots.	Average PH values.[a]	Dry weight of tops	Dry weight of roots.	Average PH values.
	Gm.	*Gm.*		*Gm.*	*Gm.*	
0.00	1.4017	0.5113	5.53	2.2010	0.4726	4.63
.01	2.1212	.7513	5.68	2.4131	1.0250	4.54
.10	3.0448	1.1837	5.68	1.9525	.3233	4.64
.25	2.6691	.8798	5.63	1.4792	.2758	4.60
.50	2.3908	.6265	5.60	2.0002	.3712	4.64
.75	2.7478	.8182	5.59	1.1501	.2227	4.63
1.00	2.2148	.6210	5.59	1.1937	.2263	4.60
1.50	3.1924	1.1366	5.61	1.3032	.2543	4.64
2.00	2.4451	.7448	5.58	1.3113	.2354	4.63
3.00	2.5870	.7962	5.55	.9408	.1829	4.56
5.00	2 2296	.5558	5.51	.8288	.1608	4.53

[a] These values represent the average of all the determinations made for each solution at the end of the growth intervals throughout the experiment period. The initial PH values of all the solutions in each series were approximately 4.7.

As indicated by the graphs of figure 7 the yields of tops and roots from the cultures containing ammonium sulphate (series D), not including the yield from the first culture in the series, were always much inferior to the corresponding yields from the Tottingham solutions. The first three cultures show a rapid decline in yield with increasing small amounts of iron, after which the decrease in yields is less marked, although there is a general decrease in the yields of both tops and roots with increasing amounts of iron throughout the series, as the gradual downward slope of the graphs indicates. This is in entire agreement with the general appearance and condition of the plants as already described, with respect to the evidence of a toxic influence as indicated by the apparent size, color, and the generally depressed condition of the plants which was progressively more pronounced with increasing amounts of iron.

The graphs representing the yields from the Tottingham solutions (series C), on the other hand, show that in these solutions the variations in the amounts of iron within the range here used (0.01 mgm. to 5.0 mgm. per liter of solution) did not produce marked differences in the yields. All the solutions of this series produced relatively high yields, and there were no evidences of specific toxicity, although, as previously stated, the 0.01–mgm. and the 0.10–mgm. portions of iron were not sufficient to prevent chlorosis in the plants grown in these solutions.

It will be observed that the control culture (without iron) in the ammonium-sulphate series (series D) produced a much higher yield than did the control culture in the Tottingham series (series C). This agrees with the general appearance of the plants in these two cultures, the plants in the former being green and apparently healthy while those in the latter were chlorotic. The large difference in the dry-weight yields of the plants from these control cultures may possibly be accounted for by the fact that the magnesium-sulphate and monopotassium-phosphate crystals contained small amounts of iron (less than 0.001 per cent by analysis) as an impurity. This very small trace of iron was perhaps soluble in the solution containing ammonium sulphate and available to the plants and insoluble and, therefore, unavailable to the plants in the Tottingham solution. This is offered only as a suggestion and may not explain the real cause of the large difference in dry top yields, since the difference in the root yields from these control cultures is very slight.

In this connection it might be suggested that the toxic influence of the iron in the solutions containing ammonium sulphate is perhaps directly related to the hydrogen-ion concentrations of the culture solutions. The initial hydrogen-ion exponents of the solutions used in series C and D are approximately the same (P_H 4.7); but the hydrogen-ion concentrations of the Tottingham solutions change rather rapidly toward the neutral point when in contact with the plant roots, while under the same conditions the hydrogen-ion concentrations of the solutions containing ammonium sulphate are increased, or at least maintained at their initial values, during the several growth intervals between successive solution changes throughout the experiment period as is indicated by the data in Table VIII. Since iron compounds are less soluble in culture solutions with lower hydrogen-ion concentrations, it is possible that in the Tottingham solutions with decreasing concentrations of these ions sufficient iron was perhaps removed from solution by precipitation to prevent the toxic influence of this element. The suggestion here made appears to gain some support from actual observation. Precipitates invariably occurred in both the Tottingham solutions and the solutions containing ammonium sulphate, in which the proportions of iron as here used were relatively high, after standing in contact with the plant roots; but these precipitates always appeared first in the Tottingham solutions and were always much more pronounced in these than they were in the solutions containing ammonium sulphate.

Experience has, of course, shown that culture solutions may not be expected to produce good growth of green plants if they contain more than a very small quantity of available iron, and observations of toxicity produced by iron under various experimental conditions are frequent in the literature. Thatcher (*16*) makes the broad statement that—

> Only soluble ferric compounds seem to serve as a suitable source of supply of the element; ferrous compounds being usually highly toxic to plants.

In this connection, Hartwell and Pember (*7*) found ferrous sulphate to be toxic to barley and rye seedlings when added to Knop's solution in concentrations of *N/5,000*. This concentration, however, is much higher than the concentrations of ferrous sulphate used in the present experiments. These authors state that the absolute effect of a given strength of ferrous sulphate would be expected to depend upon the frequency of the renewed applications and upon the nature of the nutrient solution employed. Ruprecht (*14*) also found that ferrous sulphate when present in culture solutions above four parts per million of iron exerts a toxic effect upon clover seedlings.

From a consideration of the data presented in the preceding pages it is evident that the form and quantity of iron in a medium for plant growth is a very important factor. In experiment I of this study the iron supplied to the plants in the form of ferric phosphate was entirely inadequate to support plant growth in the Tottingham solutions. When, however, iron in this form was supplied to the plants in the solutions containing ammonium sulphate which was substituted for the potassium nitrate in the Tottingham solutions, iron did not become a limiting factor for growth and these solutions produced excellent plants which were vigorous and healthy throughout the early stages of growth extending over a period of five weeks. On the other hand, when iron was supplied in the form of the soluble ferrous sulphate, the Tottingham solutions supported excellent growth and produced very high yields of both tops and roots, while the solutions containing ammonium sulphate were exceedingly toxic to the plants, and this toxicity was intensified by the use of higher concentrations of the iron salt but disappeared entirely when only a small trace of iron (0.01 mgm. per liter) was supplied. It appears, therefore, that the injury to the plants did not result from the direct influence of the ammonium salt here used but was caused by the iron which was made available in excessive quantities through the presence of the ammonium salt.

YIELDS OF TOPS AND ROOTS IN RELATION TO SALT PROPORTIONS

The relation between yields and salt proportions will here be considered for two series only, these being the Tottingham series (A) of experiment II and the ammonium-sulphate series (B) of experiment I. In these two series the plants were at all times healthy and vigorous and suffered no apparent injury. The plants of the Tottingham series (A)

of experiment I and those of the ammonium-sulphate series (B) of experiment II were greatly restricted in the growth rates, as already explained. The yields from these two series will, therefore, not be considered in relation to the salt proportions of the culture solutions.

The relative yield values as given in Table III for the six cultures producing the highest yields in each of the two series here to be considered were plotted on the tetrahedral diagram like that employed by Tottingham (*18*) but here presented in perspective in somewhat the same manner as was done by Espino (*3*). Since the low and medium yields have little interest in this connection, they will be omitted from the diagrams and discussion. The high yields of tops for both series were plotted

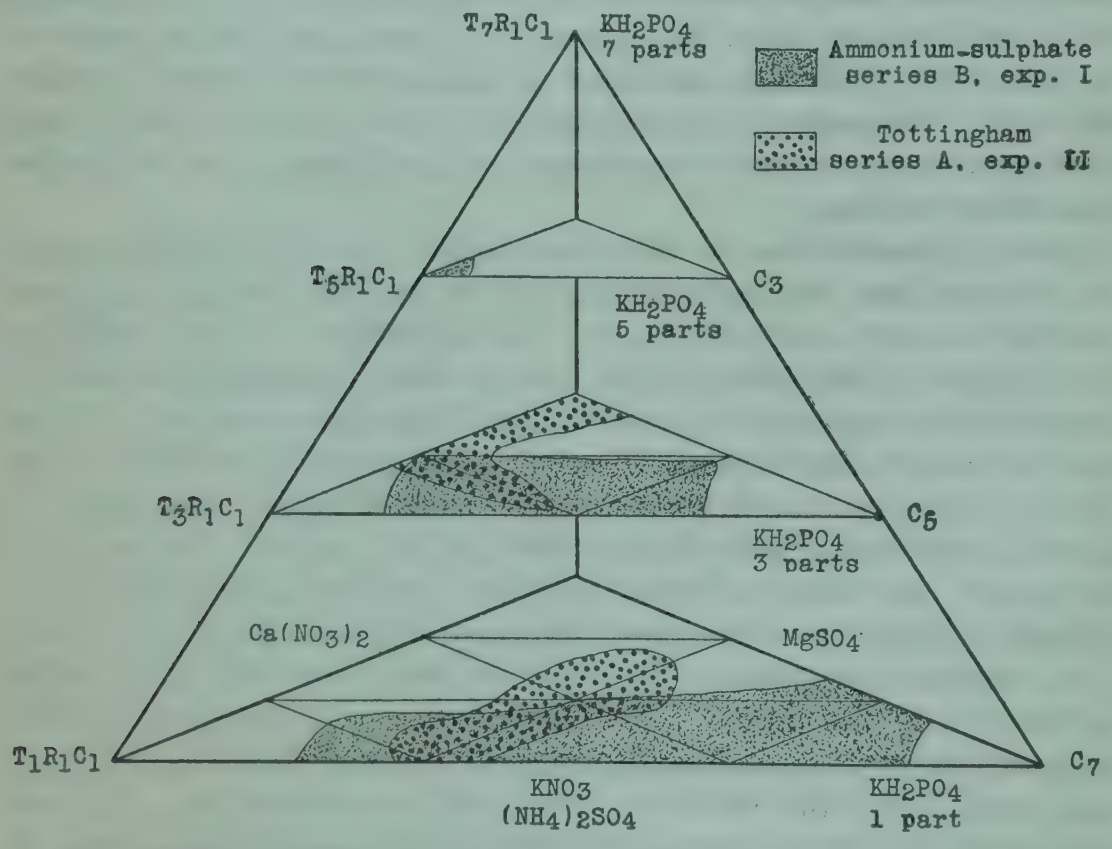

FIG. 8.—Distribution of relative dry-weight values of tops for the best six cultures in each series.

on a single diagram, as were also the corresponding yields of roots for the two series. The areas representing the high yields of the two series are distinguished by shading. On the diagram of figure 8 the dotted areas represent the yields of tops from the best six cultures of the Tottingham series (series A of experiment II), and the stippled areas represent the corresponding yields from the best six cultures of the ammonium-sulphate series (series B of experiment I). The relative dry-weight yields of roots from the best six cultures of each of the two series are similarly represented on the diagrams of figure 9.

Comparing now the areas representing the high yields of tops from the Tottingham series with those representing the corresponding yields from the ammonium-sulphate series, as shown on the diagram of figure 8, it

will be observed that there is considerable overlapping of these areas. Out of the group of six high-yielding cultures in each series three are corresponding cultures and are included in the areas marking high yields of tops in both series. These three cultures are $T_1R_1C_3$, $T_1R_3C_3$, and $T_3R_3C_1$. The maximum yield from the Tottingham series was produced by culture $T_1R_3C_3$, while the maximum yield from the ammonium-sulphate series occurred with culture $T_1R_1C_5$. A similar comparison of the areas of high root yields on the diagram of figure 9 also shows a certain amount of overlapping of the areas representing these yields from the two series under consideration. The three cultures $T_1R_1C_3$, $T_1R_3C_5$, $T_3R_1C_3$ are included in the areas representing high root yields in both series. The maximum yields of roots were produced by cultures $T_1R_3C_5$

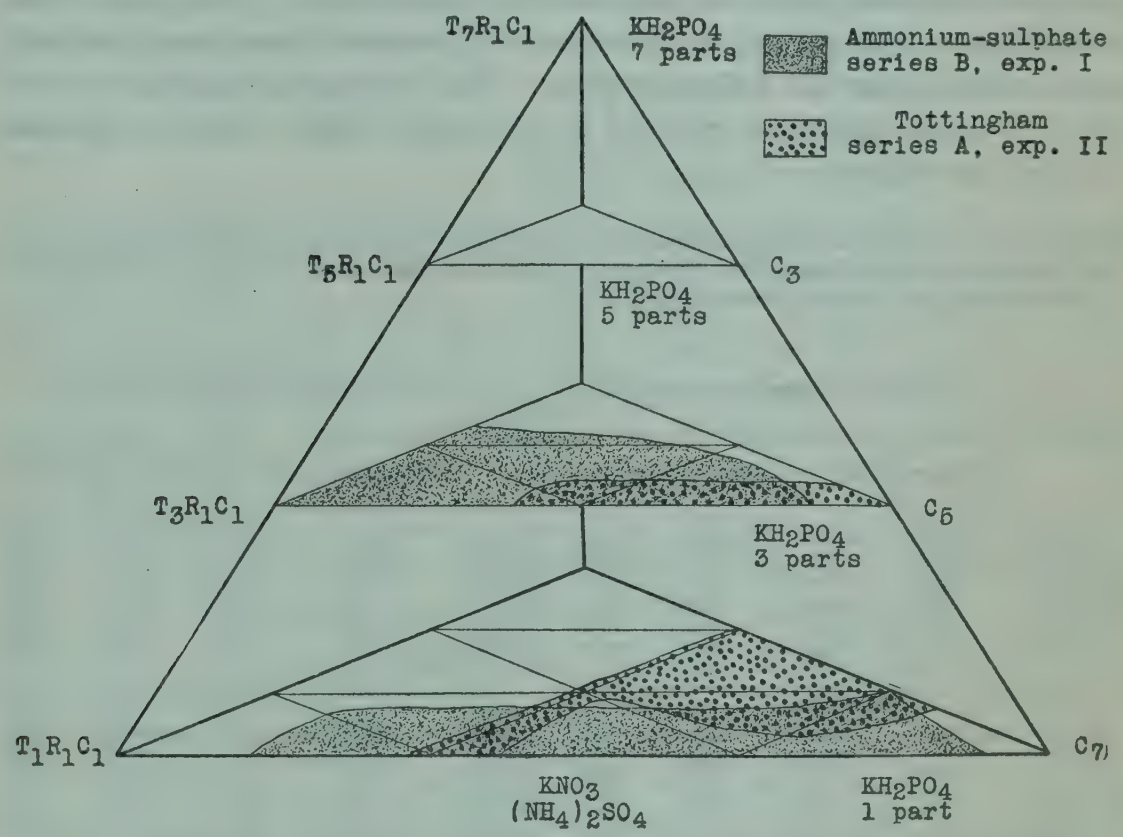

FIG. 9.—Distribution of relative dry-weight values of roots for the best six cultures in each series.

and $T_1R_1C_3$ in the Tottingham series and the ammonium-sulphate series, respectively. In each of these series, however, the maximum yield of tops and that of roots was produced by different cultures.

A comparison of the diagram of figure 8 with that of figure 9 shows a pronounced similarity and very close agreement between the areas representing high yields of tops and those representing high root yields for the ammonium-sulphate series. Five of the six cultures which are included in the areas of high top yields (fig. 8) also appear in the areas of high root yields (fig. 9). In the Tottingham series, however, no such marked similarity between the areas of high top yields and those of high root yields is apparent. In this series only two cultures appear in the areas of both high top yields and high root yields, although the detached

culture $T_3R_1C_5$, marking the lower limit in the range of high top yields, appears also in an area representing high root yields.

From the distribution of the areas representing high yields of tops and roots on the diagrams of figures 8 and 9, it is at once apparent that good growth may be correlated with relatively wide ranges in the proportions of most of the salts comprised in the culture solutions of the two series here considered, but for ammonium-sulphate a narrow range only is indicated. This is brought out best by the data in Table IX, which gives the volume-molecular partial concentrations of the salts and the ranges of these for the culture solutions in each series which produced the best six yields of tops and of roots. At the bottom of this table are given the maximum and minimum partial concentrations of each salt used and the total ranges of these for the entire series. The partial concentrations of the culture solutions which produced maximum yields in each series appear in bold-face type. The cultures comprised in the table are those which are included in the areas of high yields of tops and roots on the diagrams of figures 8 and 9.

TABLE IX.—*Volume-molecular partial concentrations and ranges of these for the salts in the solutions producing the highest six yields of tops and roots in the Tottingham series and in the ammonium-sulphate series*

	Culture No.	Tottingham Series (experiment II).				Culture No.	Ammonium-sulphate series (experiment I).			
		KNO_3.	KH_2PO_4.	$Ca(NO_3)_2$.	$MgSO_4$.		$(NH_4)_2SO_4$.	KH_2PO_4.	$Ca(NO_3)_2$.	$MgSO_4$.
Tops	$T_1R_1C_3$...	0.0020	0.0021	0.0044	0.0119	$T_1R_1C_3$	0.0014	0.0021	0.0044	0.0119
	$T_1R_3C_3$...	**.0060**	**.0021**	**.0044**	**.0071**	$T_1R_1C_5$	**.0014**	**.0021**	**.0073**	**.0071**
	$T_3R_1C_5$...	.0020	.0063	.0073	.0024	$T_1R_3C_5$	.0042	.0021	.0073	.0024
	$T_3R_3C_1$...	.0060	.0063	.0015	.0071	$T_3R_1C_3$	.0014	.0063	.0044	.0071
	$T_3R_5C_1$...	.0100	.0063	.0015	.0024	$T_3R_3C_1$	.0042	.0063	.0015	.0071
	$T_5R_3C_1$...	.0060	.0106	.0015	.0024	$T_5R_1C_1$	.0014	.0106	.0015	.0071
	Range..	.0080	.0085	.0058	.0095		.0028	.0085	.0058	.0095
Roots	$T_1R_1C_3$...	.0020	.0021	.0044	.0119	$T_1R_1C_3$	**.0014**	**.0021**	**.0044**	**.0119**
	$T_1R_3C_3$...	.0060	.0021	.0044	.0071	$T_1R_1C_5$	.0014	.0021	.0073	.0071
	$T_1R_3C_5$...	**.0060**	**.0021**	**.0073**	**.0024**	$T_1R_3C_5$	.0042	.0021	**.0073**	.0024
	$T_1R_5C_3$...	.0100	.0021	.0044	.0024	$T_3R_1C_1$	.0014	.0063	.0015	.0119
	$T_3R_1C_3$...	.0020	.0063	.0044	.0071	$T_3R_1C_3$	.0014	.0063	.0044	.0071
	$T_3R_1C_5$...	.0020	.0063	.0073	.0024	$T_3R_3C_1$	.0042	.0063	.0015	.0071
	Range..	.0080	.0042	.0029	.0095		.0028	.0042	.0058	.0095
Entire series:										
	Maximum.	.0140	.0148	.0102	.0166		.0098	.0148	.0102	.0166
	Minimum.	.0020	.0021	.0015	.0024		.0014	.0021	.0015	.0024
	Range.....	.0120	.0127	.0087	.0142		.0084	.0127	.0087	.0142

Inspection of the data in Table IX shows that the salt proportions characterizing the cultures which gave the highest six yields of tops and roots in the two series are limited to certain ranges in these proportions which are always less extensive than the total ranges for the entire series. The range in the proportions of each salt which produced high yields of tops in the Tottingham series is relatively wide, in each instance being approximately two-thirds of the total range of these proportions

for the entire series. In the ammonium-sulphate series the ranges in the proportions of monopotassium phosphate, calcium nitrate, and magnesium sulphate are in absolute agreement with the corresponding ranges in the proportions of these salts in the Tottingham series, while the range in the proportions of ammonium sulphate for high yields of tops is relatively low, being only one-third of the total range in the proportions of this salt for the entire series. This range includes the lowest proportion of this salt used in the series.

The cultures which produced high root yields in the Tottingham series are characterized by relatively wide ranges in the proportions of potassium nitrate and magnesium sulphate and relatively narrow ones in the proportions of monopotassium phosphate and calcium nitrate, while high yields of roots in the ammonium-sulphate series are associated with a narrow range in the proportions of ammonium sulphate and monopotassium phosphate and a wide range in the proportions of the other two salts.

SUMMARY

The experiments described in this paper were conducted for the purpose of studying, in a comparative way, the effects of ammonium sulphate in nutrient solutions upon the growth of young wheat plants and to determine the influence of this salt upon the ability of the plants to utilize iron from a soluble ferrous salt and an insoluble ferric salt. A study was also made of the change in reaction of the nutrient solutions induced by contact with the plant roots. Two series of culture solutions were used. The first of these comprised 20 solutions selected from the Tottingham series of 84, and the second series consisted of the same solutions modified by substituting ammonium sulphate for the potassium nitrate in equivalent osmotic concentrations. All the solutions had a total osmotic concentration value of approximately 1 atmosphere. The soluble and the insoluble iron in the form of ferrous sulphate and ferric phosphate, respectively, was added to the solutions in quantities of 0.83 mgm. of iron per liter of solution. The culture solutions were renewed at regular intervals of 3½ days throughout a total growth period of 35 days.

The main results of the experiments may be summarized as follows:

(1) The plants grown in the Tottingham solutions invariably produced a marked decrease in the hydrogen-ion concentrations of the solutions.

(2) The plants grown in the solutions containing ammonium sulphate invariably increased the hydrogen-ion concentration of these solutions during the early stages of growth. During the first five weeks of growth the hydrogen-ion concentrations were maintained at a much higher level in these solutions than in the unmodified Tottingham solutions, although the initial P_H values of corresponding solutions of the two types were practically the same.

(3) The direction of the change in reaction of the culture solutions produced by the growing wheat plants was determined by the nature of the salt constituents comprised in the solutions.

(4) Ferric phosphate, in the quantities used, was not sufficiently available in the Tottingham solutions to supply the needs of the plants for iron. The low yields produced by these solutions were correlated with a high degree of chlorosis and with high P_H values. On the other hand, this form of iron appears to be readily available to the plants in the solutions containing ammonium sulphate. The high yields produced by these solutions were associated with relatively low P_H values, and the plants were entirely free from chlorosis.

(5) Ferrous sulphate, in the quantities used, was sufficiently available in the Tottingham solutions to satisfy the needs of the plants for iron. These solutions with the soluble form of iron produced excellent growth and high yields, and no chlorotic or toxic effects were apparent. The solutions containing ammonium sulphate with this form of iron in quantities of more than 0.01 mgm. of iron per liter of nutrient solution were very toxic to the plants, the degree of toxicity increasing with increase in the amounts of iron added to the solutions.

(6) The nature of the nutrient solution with respect to the salt constituents and hydrogen-ion concentration appears to determine the availability and the efficiency of a given iron salt for plant growth.

(7) The highest six yields of wheat tops from the Tottingham series are correlated with relatively wide ranges in the proportions of all four salts employed. The maximum yield of tops from these series was produced by a solution having the following salt proportions: Potassium nitrate 0.0060 M.; potassium phosphate 0.0021 M.; calcium nitrate 0.0044 M.; and magnesium sulphate 0.0071 M., with ferrous sulphate (0.83 mgm. of iron per liter) as the source of iron for the plants.

(8) The highest six yields of tops from the series of solutions containing ammonium sulphate are associated with relatively wide ranges in the proportions of potassium phosphate, calcium nitrate, and magnesium sulphate, but with a narrow range in the proportions of ammonium sulphate. The maximum yield of tops from these series was produced by a solution having the following salt proportions: Ammonium sulphate 0.0014 M.; potassium phosphate 0.0021 M.; calcium nitrate, 0.0073 M.; and magnesium sulphate 0.0071 M., with ferric phosphate (0.83 mgm. of iron per liter) as the source of iron for the plants.

(9) In the series of solutions containing ammonium sulphate, high yields of tops were generally associated with high yields of roots; but no such correlation between tops and roots existed in the Tottingham series.

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PLATE 136

A.—Ferric phosphate as the source of iron for plants, 0.75 mgm. of iron per liter of nutrient solution.

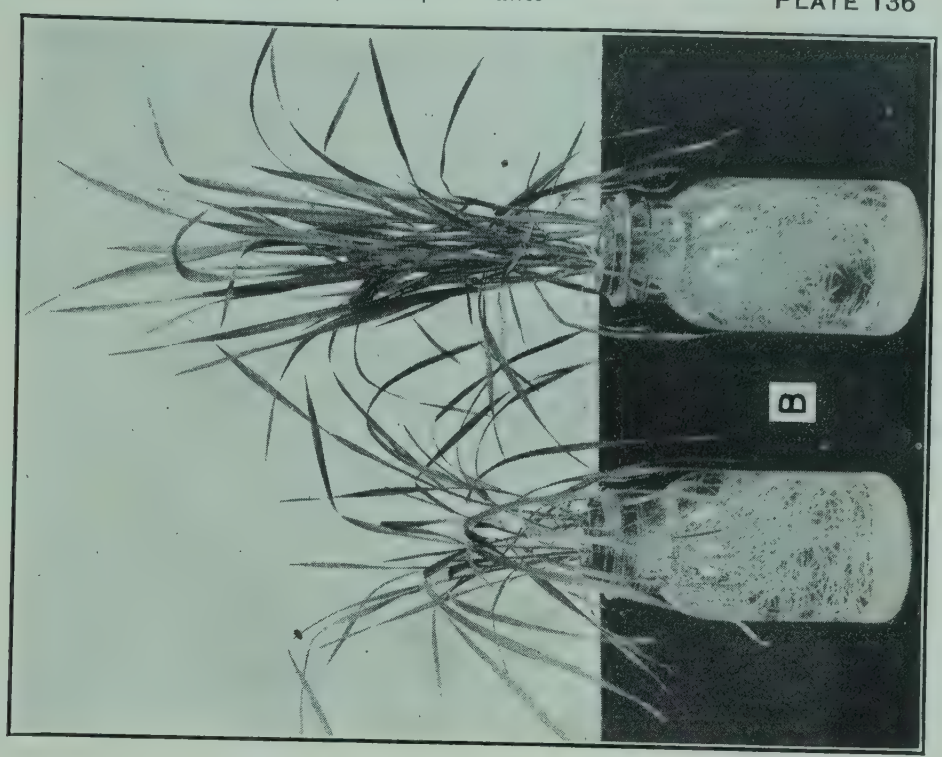
The plants in the culture on the left were grown in the Tottingham solution $T_3R_1C_5$ modified by substituting ammonium sulphate for the potassium nitrate in equivalent osmotic concentrations. The plants are green and healthy.

The plants in the culture on the right were grown in the Tottingham solution $T_3R_1C_5$ unmodified. The plants are chlorotic and much smaller than those grown in the solution containing ammonium sulphate.

B.—Ferrous sulphate as the source of iron for plants, 0.75 mgm. of iron per liter of nutrient solution.

The plants in the culture on the left were grown in the Tottingham solution $T_3R_1C_5$ modified by substituting ammonium sulphate for the potassium nitrate in equivalent osmotic concentrations. The plants are small and unhealthy.

The plants in the culture on the right were grown in the Tottingham solution $T_3R_1C_5$ unmodified. The plants are large, green, and healthy.


B

A

DISPERSION OF FLIES BY FLIGHT

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A definite knowledge of the means of dissemination of various species of economic insects is of much importance in control or eradicative undertakings. Undoubtedly the spread of injurious forms by artificial means is in general of most pronounced importance. With free-flying species, however, natural dispersion deserves careful consideration. This is particularly true of the various species of flies which directly affect man and animals. The accumulation of data on the possibilities of flight of various injurious species of flies should aid economic work in several ways.

1. It should help in the study of the spread of fly-borne diseases, either in large districts or locally.

2. It should make possible the proper location of dumps, incinerators, hog-feeding stations, and other favorable breeding grounds so that the menace to towns, cities, military camps, etc., will be reduced to a minimum.

3. In control work in restricted districts or about individual plants it should help in determining the extent to which fly-breeding grounds in the neighborhood affect these undertakings.

4. In the prosecution of large-scale control work against certain species on farms or ranges, it should show how widespread must be the effort if marked results are to be accomplished.

5. It should make it possible to determine whether control campaigns are accumulative from year to year in their effect, or whether, owing to extensive migration, the results are effective only during one year or one period.

Realizing the desirability of collecting data along this line, a number of investigators in various parts of the world have carried out dispersion tests. These were mainly with the house fly (*Musca domestica* L.), although limited numbers of a few other species were observed in the experiments. Most of these tests were conducted under more or less distinctly urban conditions. The observations and main conclusions of these various experimenters have been so well summarized by Parker [1] that they will not be repeated here. Parker also discusses rather fully in the same article the methods of marking the flies in the tests.

The maximum range of dispersion recorded up to the work of Parker was 1,700 yards. This distance was noted in a test in England carried

[1] PARKER, R. R. DISPERSION OF MUSCA DOMESTICA LINNAEUS UNDER CITY CONDITIONS IN MONTANA. *In* Jour. Econ. Ent., vol. 9, no. 3, p. 325-354, pl. 24-26. 1916.

out under urban conditions by Copeman, Howlett, and Merriman in 1911.[1]

Parker's experiments in Montana were also carried out largely under city conditions. The greatest range of dispersion shown in his tests was 3,500 yards, or nearly 2 miles, with the house fly. A record of 500 yards was made for *Muscina stabulans* Fall., and 160 yards for the black blowfly (*Phormia regina* Meig.). These last species, however, were released in comparatively insignificant numbers.

In addition to exact evidence obtained by recapturing marked and released flies, other evidence of dispersion is at hand. Hodge[2] has observed house flies, stable flies (*Stomoxys calcitrans* L.), and "blue bottles" in considerable numbers at cribs 5 and 6 miles out in Lake Erie north of Cleveland, Ohio. He concludes that the flies appear to be blown out at least 6 miles off shore. In 1917, Ball[3] presented evidence which indicates that the house fly may go distances of 45 or 95 miles. These observations were made at the Rebecca Light Shoal off the coast of Florida, and the conclusion was reached that the flies came down wind from Cuba (95 miles distant) and at times from the Marquesas Keys (24 miles distant) or possibly from Key West, Fla. (46 miles distant). While the evidence of flight over these long distances is convincing it is hardly incontrovertible. The flight in these instances was over water, at least for the most part; hence the conditions were very different from those existing under usual urban or rural conditions.

With a view to securing some definite facts as to the tendencies and possibilities of dispersion under rural conditions, a series of tests with injurious species was carried out in northern Texas during the summers of 1916 and 1918.

METHOD OF CATCHING, MARKING, AND LIBERATING FLIES

Practically the same method of handling the flies was used throughout the several tests conducted. The supply was secured by baiting large conical flytraps with "gut slime," a packing house by-product. These traps were left in operation from a few hours up to 24 hours; then a screen cylinder was placed on top of the flytrap. The cover of the trap was removed, and the flies were agitated so that they would pass upward into the cylinder, which was first supplied with a number of green branches from trees. Few of the weaker, disabled flies would pass upward into the cylinders. When several thousand had passed into the cylinder a cheesecloth was placed over the lower end of it. All the flies were

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[2] HODGE, C. F. THE DISTANCE HOUSE FLIES, BLUE BOTTLES AND STABLE FLIES MAY TRAVEL OVER WATER. *In* Science, n. s., v. 38, no. 980, p. 512–513. 1913.

[3] BALL, S. C. MIGRATION OF INSECTS TO REBECCA SHOAL LIGHT-STATION AND THE TORTUGAS ISLANDS, WITH SPECIAL REFERENCE TO MOSQUITOES AND FLIES. *In* Papers Dept. Mar. Biol. Carnegie Inst. Wash., v. 12 (Publ. 252), p. 193–212. 1918. Bibliography, p. 212.

captured in Dallas or Fort Worth at the packing houses and were then transported to the point of liberation. The time from placing the flies in the cylinders until they were liberated ranged from 1 to 2 hours.

When the point of liberation was reached the cylinders were slipped into a canvas bag one at a time and from 1 to 2 ounces of finely powdered red chalk or paint pigment were introduced into the cage, which was inverted several times. The top was then removed, and the flies were allowed to escape. All flies were then shaken from the cages.

The number and percentage of the different species of flies released were estimated by looking into the screened cylinders. While it is realized that this estimate is very rough, the writers' experience in handling great numbers of flies in traps has added to the reliability of the estimates. In one instance the flies in one trap were killed, and the proportion of species and sex was determined by actual count.

When the flies emerged from the cage they were all distinctly covered with the chalk, the majority of them being brilliant red. In one instance yellow chalk was used and in another the flies were sprayed with rosalic acid, but the yellow color was not so readily distinguished in the mass of flies caught in the recovery traps, and not a single fly sprayed with rosalic acid was identified when an alkaline solution was applied to the specimens taken in the recovery traps. In a few of the liberations a red paint pigment was used, and this also seemed fairly satisfactory.

In every case there was a considerable mortality among the flies placed in the cages, but it is believed that this was caused more by the heat while the specimens were being transported to the place of liberation than by the application of the marking agent. That there may have been some deleterious effect from the application of the chalk dust can not be denied, but the fact that some marked specimens were recovered 17 days after application indicates that the method of marking was not highly injurious.

ACTION OF FLIES WHEN LIBERATED

All liberations were made on the ground in open fields. In every instance a considerable number of the flies were observed to take to the air immediately, some passing upward to a considerable height. They seemed to fly freely in all directions, but there seemed to be more going with the wind or at right angles to it than in other directions. A large number settled on the grass near by, and many of these were observed to be preening themselves in a contented way. In experiments where trees were near at hand large numbers were observed to settle on the leaves. Many flies exhibited great thirst, as was shown by efforts to procure moisture from the leaves and perspiration on our bodies. They persistently stuck to our clothing, hands, and faces, and, although the conveyance used was always left some distance from the point of liberation, a few flies were in every instance observed to be

about the vehicle when the start away from the point was made. The flies were at first driven off, and after a short distance had been traveled all of those about the vehicle were killed. A second and sometimes a third examination were made to make sure that no colored specimens were following.

RECOVERY AND IDENTIFICATION OF FLIES

In order to determine the distance of dissemination, conical traps 18 inches in diameter baited with "gut slime" were set at measured distances in different directions from the point of liberation. The flies collected in these recovery traps were killed at daily intervals as nearly as possible, and the mass of flies was carefully gone over for colored specimens. In most instances the colored flies could be identified with the unaided eye, but when any doubtful specimens were found they were examined with a microscope and the presence of particles of chalk could then be determined accurately. During the first few days after liberation most of the flies were strongly colored, but later the specimens retained the color mainly on the halteres.

The sex and species of the marked flies recovered were determined, and in many cases the percentage of the different species of unmarked flies in the recovery traps was estimated by examination of a certain number of flies, and in the same way the sexes were estimated in a number of instances. The weight of the catch in each recovery trap was also determined.

Owing to the different conditions under which the several dissemination tests were made it seems best to discuss them separately.

INITIAL FLIGHT TEST NEAR FORT WORTH, TEX.

On May 31, 1916, at 1.30 p. m., 7,000 or 8,000 flies were marked with red chalk and liberated in an open field about ½ mile east of the two large packing houses which are located approximately 2 miles north of Fort Worth. One of the prime objects of this test was to determine the freedom with which the flies would go toward packing houses from surrounding areas where more or less attractive feeding and breeding conditions for flies of all kinds occurred. Eighteen recovery traps were used in this test. They were set at distances and directions as follows from the point of liberation (fig. 1): Trap No. 1, 930 feet 15° north of west on dock of a silo construction company; trap No. 2, 851 feet 15° south of west on a platform of a plant where dead animals were rendered, hence very attractive for flies; trap No. 3, 1,037 feet southwest near a small slaughterhouse with surroundings attractive for flies; trap No. 4, 1,323 feet 12° south of west in garden behind store; traps No. 5, 6, 7, and 8, about 2,123 feet 25° south of west located around a rendering plant where conditions were attractive to flies; traps No. 9, 10, 11, 12, and 13, about 2,588 feet 5° south of west and within the inclosure of one of the large

packing houses; traps No. 14 to 18, inclusive, 3,076 feet 5° north of west within the inclosure of another large packing house. To make traps No. 2 and 3 still more attractive to blowflies a large amount of meat waste was hauled out into the field between them and partially plowed under.

A creek fringed with trees flows nearly north between the point of liberation and the packing houses, about 1,100 feet from the point of liberation; thus the flies, in order to reach traps No. 4 to 18, inclusive, would have to cross this stream. The closest road with any considerable amount of travel is about 1,400 feet from the point of liberation, passing near traps No. 5 to 13 and turning north at right angles near trap No. 4.

Almost immediately after release two colored screw-worm flies (*Chrysomya macellaria* Fab.) were observed at trap No. 1, and several were seen about the rendering plant at trap No. 2. About three hours after liberation a marked *C. macellaria* was observed through the screen in trap No. 5.

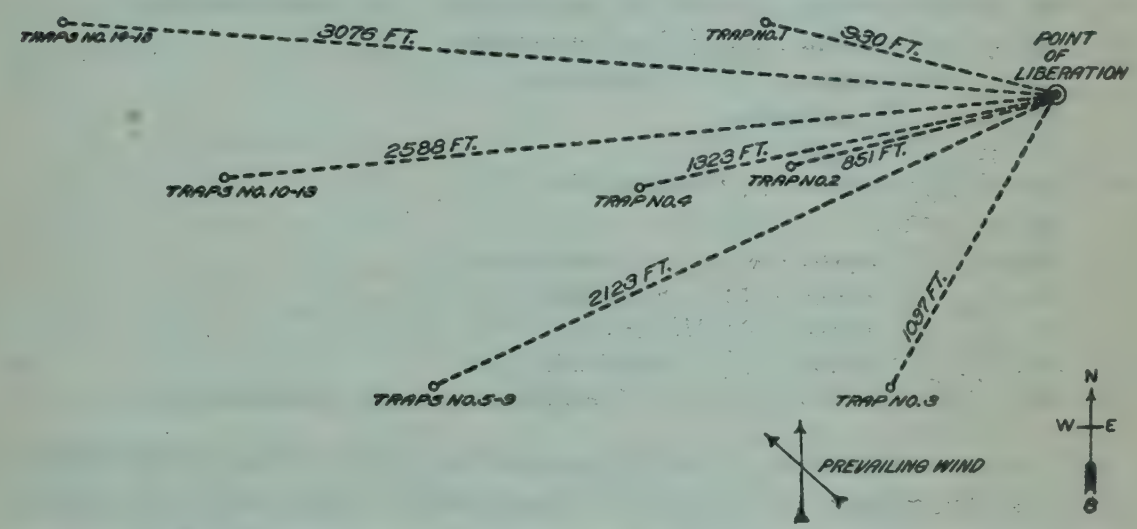


FIG. 1.—Diagram of dispersion test at Fort Worth, Tex., indicating relation of recovery traps to point of release.

June 1, 11.45 a. m., flies in traps No. 10 to 13, inclusive, were killed and examined and the following marked flies found: Trap No. 10, 1 ♀ *Phormia regina* (the black blowfly), 1 ♀ *Chrysomya macellaria;* No. 11, 1 ♀ *P. regina;* No. 12, 1 ♀ *P. regina;* No. 13, 2 ♀ *P. regina*, 1 ♀ *C. macellaria.* June 1, between 2 and 3.45 p. m., the following colored flies were found in traps No. 1 to 9, inclusive: No. 1, 2 ♂ and 16 ♀ *P. regina*, 1 ♀ *C. macellaria*, 5 ♀ *Musca domestica* (house fly); No. 2, 11 ♀ *P. regina*, 1 ♀ *M. domestica;* No. 3, 1 ♂ and 5 ♀ *P. regina*, 3 ♀ *C. macellaria*, 5 ♀ *M. domestica;* No. 4, 2 ♂ and 9 ♀ *P. regina*, 2 ♀ *C. macellaria*, 2 ♀ *M. domestica;* No. 5, 6 ♀ *P. regina*, 3 (2 ♀, 1?) *C. macellaria.* No. 6, 7, 8, 9, none. June 2, 9.45 to 11.20 a. m., No. 14 to 18 contained the following colored flies: 4 ♀ *P. regina*, 2 ♀ *C. macellaria*, 1 ♀ *M. domestica.* June 2, 12.30 to 2 p. m., flies in No. 5 to 13, inclusive, were killed and examined, and the following marked specimens were found: No. 5, 4 ♀ *P. regina*, 1 ♀ *C. macellaria;* No. 6, 1 ♀ *M. domestica;* No. 7, none; No. 8, 2 ♀ *P. regina;* No. 9, none;

No. 10, 1 ♂, 1 ♀ *P. regina;* No. 11, none; No. 12, 1 ♀ *M. domestica;* No. 13, none. June 2, 2.30 p. m., No. 1, 1 ♀ *P. regina,* 1 ♀ *C. macellaria;* No. 2, 5 ♀ *P. regina,* 1 ♀ *C. macellaria* (sex ?), 2 ♀ *M. domestica;* No. 3, 2 ♀ *P. regina,* 1 ♀ *M. domestica;* No. 4, 2 ♀ *P. regina;* No. 5, none. June 3, 9.30 to 11.20 a. m., No. 5, none; No. 6, 1 ♀ *M. domestica;* No. 7, 2 ♀ *P. regina;* No. 8, 9, and 10, none; No. 11, 1 ♀ *C. macellaria;* No. 12, 1 ♀ *P. regina;* No. 13, none. June 3, 1.30 p. m., No. 1, none; No. 2, 1 ♀ *C. macellaria;* No. 3, 1 ♀ *P. regina;* No. 4, 1 ♀ *C. macellaria.* June 5, 10 a. m., No. 1 and 2, none; No. 3, 1 ♀ *P. regina;* No. 4, 3 ♂ *P. regina;* No. 5, 1 ♀ *P. regina;* No. 6 and 7, none; No. 8, 1 ♀ *C. macellaria;* No. 9 to 14, inclusive, none.

The grand total of all the colored flies caught in the traps was 126, distributed among the traps as follows:

No. 1, 26 (*P. regina* 2 ♂, 17 ♀; *C. macellaria* 2 ♀; *M. domestica* 5 ♀).
No. 2, 21 (*P. regina* 16 ♀; *C. macellaria* 1 ♀, 1 ?; *M. domestica* 3 ♀).
No. 3, 19 (*P. regina* 1 ♂, 9 ♀; *C. macellaria* 3 ♀; *M. domestica* 6 ♀).
No. 4, 19 (*P. regina* 2 ♂, 14 ♀; *C. macellaria* 3 ♀).
No. 5, 15 (*P. regina* 11 ♀; *C. macellaria* 3 ♀, 1 ?).
No. 6, 2 ♀ *M. domestica.*
No. 7, 2 ♀ *P. regina.*
No. 8, 3 (*P. regina* 2 ♀; *C. macellaria* 1 ♀).
No. 9, none.
No. 10, 4 (*P. regina* 1 ♂, 2 ♀; *C. macellaria* 1 ♀).
No. 11, 2 (*P. regina* 1 ♂; *C. macellaria* 1 ♀).
No. 12, 3 (*P. regina* 2 ♀; *M. domestica* 1 ♀).
No. 13, 3 (*P. regina* 2 ♀; *C. macellaria* 1 ♀).
No. 14 to 18, 7 (*P. regina* 4 ♀; *C. macellaria* 2 ♀; *M. domestica* 1 ♀).

It is noteworthy that while the percentage of the three species recovered was estimated at the time of liberation to be *Musca domestica* 7, *Chrysomya macellaria* 70, *Phormia regina* 22, the percentage of these species as recovered was approximately *M. domestica* 14, *C. macellaria* 16, *P. regina* 70. At first sight this might lead one to think that *P. regina* was more prone to migration than *C. macellaria;* however, when we group the first four traps, which were from 930 to 2,588 feet from the point of liberation, and the last 14, which were from 2,123 to 3,076 feet, exactly 50 per cent of the total number of marked *C. macellaria* recovered were in the more distant traps while the percentage of marked *P. regina* was but slightly over 30.5. Comparing the house flies in the same way, about 22 per cent were taken in the more distant group of traps. Attention might also be called to the fact that of the 126 marked flies recovered only 6, or 4.75 per cent, were males. The percentage of the sexes of the flies liberated was not determined, but in the usual catch the number of males is much greater than this. Five of these males were taken in the four nearest traps, which might further suggest that the tendency to distant dissemination is more marked in the females.

It is notable that over 64 per cent of the total number of marked flies recovered were found to be captured when the traps were emptied about 24 hours after the liberation had been made, and several of the traps were not examined within this 24-hour period.

This test also shows that distances up to 1,000 feet are covered in a few minutes by flies, that the screw-worm fly may travel nearly half a mile in 3 hours, and that the house fly may go over 3,000 feet in less than 48 hours.

Since the recovery traps were not placed in all directions from the point of liberation, it is very difficult to draw conclusions in this test in regard to the effect of wind on dissemination. During this recovery period the wind during the daytime varied in velocity from 5 to 30 miles, and the prevailing directions were south and southeast. (Table I.) The wind with a velocity of from 13 to 24 miles blew from the southeast and south during the 48 hours following release, and since the majority of the marked flies recovered were taken within that period it is seen that those captured traveled practically at right angles to this wind.

A second liberation of about 10,000 flies marked with yellow chalk was made at 4.30 p. m., June 1. The proportion of the different species was estimated to be as follows: *Chrysomya macellaria* 60 per cent, *Phormia regina* 28 per cent, *Musca domestica* 10 per cent, other species, including Lucilia, Ophyra, Sarcophaga, and *Muscina stabulans*, 2 per cent. Difficulty was encountered in identifying the flies from this liberation. None were observed except on June 2, when the following recoveries were positively made: No. 14, 10.15 a. m., 1 ♀ *P. regina;* No. 10, 12.30 p. m., 1 ♀ *P. regina;* No. 11, 2 ♀ *P. regina*, 1 ♀ *C. macellaria;* No. 13, 1 ♂ *P. regina*, 1 ♀ *C. macellaria;* No. 1, 1.30 p. m., 2 ♂ and 1 ♀ *P. regina;* No. 2, 1 ♀ *C. macellaria.* This shows that *P. regina* traveled over 3,000 feet in less than 18 hours after release and that night hours were included in this period.

TABLE I.—*Climatological data relating to initial dispersion test at Fort Worth, Tex.*

Date.	Temperature.			Wind.		Rain.	Humidity.			Actual sun.
	Max.	Min.	Mean.	Direction during successive hours from 6 a. m. to 8 p. m.	Velocity during day.		Max.	Min.	Mean.	
1916.	° *F.*	° *F.*	° *F.*		*Miles.*	*Inch.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
May 31....	94	70	82	14 SE.....	8 to 24	0	76	39	56	92
June 1....	95	72	84	14 S.....	15 to 24	0	75	34	54	79
2....	96	75	86	2 S., 4 SW., 2 NW., 3 W., 3 NW.	5 to 21	0	76	43	60	87
3....	94	72	83	2 NE., 9 SE., 1 S., 2 SE.....	5 to 13	0	70	49	60	80
4....	87	75	81	14 SE.....	8 to 26	0	77	50	64	52
5....	86	70	78	2 S., 5 SW., 5 W., 2 NW.....	19 to 30	0	68	39	54	73

FIRST DISPERSION TEST NEAR DALLAS, TEX.

On June 29, 1916, about 18,000 flies, consisting of approximately 70 per cent *Musca domestica*, 25 per cent *Chrysomya macellaria*, 1 per cent *Phormia regina*, and 4 per cent made up of *Lucilia sericata* Meig., *Muscina stabulans*, *Sarcophaga* spp., and *Ophyra* spp. were liberated. These flies were marked just before liberation with red chalk in the manner previously described. The point chosen for liberation was in the country about 4 miles east of the edge of the city of Dallas and within a few hundred feet of a large orphanage. Sixteen recovery traps baited with "gut slime" were set along the roads leading approximately east and west and north and south from the point of liberation. Four traps were set in each direction, the outer ones being between 2 and 3 miles from the point of liberation. The distance of each trap from this point is shown in Table II. The country covered by this radius might be described as a rolling, black land, farmed area. In general, farmhouses were located from ½ to 1 mile apart and most of them maintained barns and pigpens. The country was practically open, there being only a few narrow strips of woods in the area covered. The orphanage, with a number of dwellings and two or three stores at the corner, all near the point of liberation, might be considered the most attractive point for house flies in the area.

The environment of the traps undoubtedly affects the character of the entire catch and also the tendency for flies in their dissemination to linger near them and perhaps ultimately be captured. For this reason the surroundings of each trap are briefly given.

West, No. 1, behind small grocery store, some refuse, no barns near; No. 2, under tree 50 yards from dairy barn and pen; No. 3, under tree near farmhouse, a few head of stock kept near; No. 4, by side of dairy barn, favorable conditions for house flies. North, No. 1, in old pigpen near farmhouse; No. 2, in weeds near road and trees, farmhouse 75 yards away; No. 3, in cornfield, about ⅓ mile from any farmhouse, no attractive places near; No. 4, near farmhouse and barns, about ¾ mile south of the village of Reinhardt. East, No. 1, under shed at gin, horses in lot and farm about 50 yards away; No. 2, in edge of cornfield, no buildings nearer than ⅓ mile; No. 3, in tall grass near small stream, nearest building about $^1/_5$ mile; No. 4, in old barnyard, numerous livestock in adjacent lot. South, No. 1, in cornfield ⅓ mile from southernmost buildings of Orphans' Home, no others nearer; No. 2, under shed by grocery store, several houses and Texas & Pacific Railway near; No. 3, in weeds in garden, farmhouse 40 yards away and several others in vicinity; No. 4, under tree, farmhouse and barns 50 yards south.

The pike road running east and west from the point of liberation is heavily traveled by all kinds of vehicles, but especially automobiles. The road to the south has a moderate amount of travel and the road to

the north light travel. Freighting trucks and dairy wagons pass on the east and west road.

On June 30, at 11.00 a. m., a second liberation consisting of about 25,000 flies marked with red chalk was made at the same point as the first. The species in this lot were in about the same proportions as those in the first release, with possibly somewhat fewer house flies and a few more screw-worm flies and *Phormia regina.*

Immediately after these flies were liberated the flies in the recovery traps were killed and removed for examination, and these examinations were made at approximately daily intervals up to and including July 11, with the exception of July 2, 4, and 9. The bait pans were replenished each day that the flies were removed from the traps.

It is interesting to note that the proportion of the species among the marked flies recovered is very similar to the proportion of those species among the flies released. These figures are given for comparison: *Musca domestica,* 70 per cent released, 77 per cent recovered; *Chrysomya macellaria,* 25 per cent released, 21 per cent recovered; *Phormia regina,* 1 per cent released, 0.83 per cent recovered; *Lucilia* spp., *Ophyra* spp., *Sarcophaga* spp., *Synthesiomyia brasiliana* Brauer and Bergenstamm, 1 per cent released, 1.16 per cent recovered. It should be remembered that the percentages of the species released are only a rough estimate. The percentage of the different species in the total catch during the recovery period was not determined; hence it can not be compared with the marked flies released and captured.

DISTANCE OF DISPERSION

The essential data in these experiments as regards the number of flies recovered in traps set at different distances in different directions are presented in Table II. It will be noted that in general the closer rings of traps caught more flies than those at greater distances, although in the outside circle of traps the number of flies taken was greater than in the third ring. This may undoubtedly be explained, at least partially, on the basis of the more advantageous positions where some of the traps in the fourth circle were set. The influence of immediate environment on the recovery of marked flies will be discussed under some of the subsequent experiments.

TABLE II.—*First dispersion test at Dallas, Tex. Species and sex of marked flies recovered in rings of traps about point of liberation*

Trap No.	Distance from place of release.	*Musca domestica.*		*Chrysomya macellaria.*		*Phormia regina.*		*Lucilia sericata.*		*Lucilia caesar.*		*Ophyra leucostoma.*		*Synthesiomyia brasiliana.*		*Sarcophaga* spp.		Undetermined.		Total.		Grand total.
	Miles.	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
1 W	0.4	165	472	61	64	1	0	0	2	0	0	0	4	0	0	0	5	0	2	227	549	776
1 N	.5	124	1,082	53	122	1	2	0	6	0	0	1	4	0	2	0	0	0	0	179	1,218	1,397
1 E	.5	65	932	1	13	0	2	0	4	0	1	0	0	0	1	0	2	0	0	66	955	1,021
1 S	.5	3	146	24	40	0	4	0	1	0	0	0	2	0	0	1	1	0	0	28	194	222
Total		357	2,632	139	239	2	8	0	13	0	1	1	10	0	3	1	8	0	2	500	2,916	3,416
2 W	1.0	11	59	36	64	0	4	0	1	0	0	0	0	0	0	0	3	0	1	47	132	179
2 N	1.1	2	34	25	71	0	3	0	0	0	1	0	1	0	0	0	0	0	0	27	110	137
2 E	1.2	2	27	3	16	0	2	0	0	0	0	0	0	0	0	0	1	0	0	5	46	51
2 S	1.2	6	153	1	6	0	0	0	1	0	0	0	0	0	0	0	0	0	0	7	160	167
Total		21	273	65	157	0	9	0	2	0	1	0	1	0	0	0	4	0	1	86	448	534
3 W	1.6	7	55	21	57	1	8	0	0	0	1	0	0	0	0	0	1	0	0	29	122	151
3 N	1.8	0	31	6	56	0	2	0	0	0	0	0	0	0	0	0	0	0	0	6	89	95
3 E	2.0	0	7	19	38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	45	64
3 S	2.0	1	11	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	26	27
Total		8	104	46	166	1	10	0	0	0	1	0	0	0	0	0	1	0	0	55	282	337
4 W	2.1	14	106	17	44	1	0	0	0	0	0	0	0	0	0	0	0	0	1	32	151	183
4 N	2.3	3	49	10	45	2	5	0	0	0	0	0	1	0	0	0	1	0	0	15	101	116
4 E	3.5	1	25	6	15	0	0	0	0	1	0	0	0	0	0	0	0	0	0	8	40	48
4 S	3.1	2	9	2	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	4	14	18
Total		20	189	35	108	3	6	0	0	1	0	0	1	0	0	0	1	0	1	59	306	365
Grand total		406	3,198	285	670	6	33	0	15	1	3	1	12	0	3	1	14	0	4	700	3,952	4,652

DIRECTION OF DISPERSION

The data obtained in regard to the direction of flight were interesting. The total number of marked flies recovered in the different directions from the point of liberation was as follows: West, 1,289; north, 1,745; east, 1,184; south, 434. Of course the different species should really be discussed separately in treating of the direction or distance of dispersion; the figures show, however, that for some reason the liberated flies of all species failed to be recovered in the traps south of the point of liberation in numbers in proportion to those recovered in the other directions. Considering the house fly and screw-worm fly in regard to the numbers traveling in different directions, it will be seen in the case of the house fly that 889 (24.66 per cent) were recovered in the west traps, 1,325 (36.76 per cent) in the north, 1,067 (29.38 per cent) in the east, and 331 (9.18 per cent) in the south. In the case of the screw-worm fly 364 (38.11 per cent) were recovered in the west, 388 (40.62 per cent) in the north, 111 (11.62 per cent) in the east, and 92 (9.63 per cent) in the south traps. In an attempt to correlate these figures with the direction of the wind it appears that there is a tendency exhibited in this test for both of these species to go with the wind rather than against it, as will be seen by referring to climatological data in Table III. The prevailing wind during the first five days was from the south and east.

The fact that more house flies were recovered in the east traps than in the west is not quite in accord with the idea that they may have traveled largely with the wind, since there was no west wind but a considerable amount of east wind. With *Chrysomya macellaria*, however, there were less than a third as many in the east traps as in the west, the north traps caught the greatest number, and the south traps less than one-fourth as many as were caught in the north. The fact that the wind was more or less choppy throughout the period makes it very difficult to draw any conclusions on its exact influence on dispersion. Numerous other factors which must be considered also tend to make the drawing of conclusions on this point hazardous.

If we attempted to account for the greater catch of flies in certain lines of traps over others by the traffic along the highways we would expect to find the greatest dispersion east and west, probably greater to the west, and about equal to the north and south, but such was not the case.

Considering the possibility of the attraction of feeding and breeding places or volume of odors, we would expect, at least for *Musca domestica*, the greatest movement westward toward the city of Dallas, but the figures do not accord with this idea. It is probable that the proximity of smaller centers of attraction, such as farmhouses, barns, etc., are more effective in influencing the spread than more distant but larger attractive areas. These conditions, however, were apparently quite similar in each direction. As *Chrysomya macellaria* and *Phormia regina* are

normally nondomestic, we would not expect the same distribution of these species as of the house fly, yet the proportions caught in different directions are very similar.

TABLE III.—*Climatological data relating to first and second dispersion tests at Dallas, Tex.*

Date.	Temperature.			Wind.		Rain.	Humidity.			Actual sun.
	Max.	Min.	Mean.	Direction during successive hours from 6 a. m. to 8 p. m.	Velocity during day.		Max.	Min.	Mean.	
1916.	°F.	°F.	°F.		Miles.	Inch.	Per cent.	Per cent.	Per cent.	Per cent.
June 29	89	70	80	4 SE., 10 E	7 to 14	0	87	55	71	100
30	93	74	83.5	10 S., 4 E	10 to 13	0	85	47	66	88
July 1	93	70	81.5	3 SE., 3 S., 2 SW., 2 E., 4 SE	4 to 10	0	84	40	62	97
2	92	73	82.5	2 E., 12 SE.	2 to 8	0	80	48	64	72
3	93	72	82.5	2 SE., 3 S., 4 SE., 5 E	3 to 13	0	78	44	61	90
4	94	73	83.5	2 E., 1 S., 1 SW., 5 E., 2 SW., 3 E.	2 to 15	.07	83	47	65	67
5	92	74	83.0	3 N., 8 NE., 3 N	5 to 14	0	77	49	63	73
6	93	74	83.5	2 NW., 3 N., 6 NW., 3 N	4 to 10	0	68	36	52	86
7	96	75	85.5	3 W., 2 N., 4 NW., 5 N	2 to 12	0	78	45	61	97
8	96	73	84.5	2 N., 5 E., 5 N., 2 NE	2 to 11	0	72	36	54	93
9	96	73	84.5	6 NW., 3 W., 5 NE	3 to 10	0	74	29	51	89
10	99	74	86.5	2 NW., 4 SE., 5 W., 3 E	2 to 7	0	74	24	49	100
11	98	77	87.5	2 SE., 4 SW., 5 SE., 3 S	2 to 11	T	69	30	49	86
12	95	78	86.5	13 S., 1 SE	5 to 13	0	68	34	51	78
13	95	77	86.0	2 SE., 12 S	4 to 11	0	63	41	52	71
14	95	78	86.5	2 S., 3 SW., 2 S., 7 SE	5 to 11	0	64	38	51	75
15	97	77	87.0	2 SW., 3 W., 1 S., 8 SE	3 to 7	0	68	43	55	89
16	101	77	89.0	2 NW., 3 W., 4 S., 5 SE	4 to 14	0	62	33	47	74
17	97	75	86.0	2 E., 1 SE., 1 S., 10 E	2 to 11	0	85	48	66	79
18	98	77	87.5	1 E., 6 SE., 1 S., 6 SE	4 to 10	0	85	35	60	89
19	99	76	87.5	4 SE., 3 E., 1 SE., 6 E	5 to 14	0	80	36	58	84
20	95	76	85.5	3 NE., 5 NW., 3 W., 3 N	5 to 12	T	80	49	64	60
21	95	76	85.5	5 NE., 1 E., 3 N., 5 NE	5 to 11	T	79	50	64	65
22	97	75	86.0	3 NW., 1 SW., 2 N., 8 NE	3 to 11	0	78	38	58	100
23	97	79	88.0	9 E., 5 NE	3 to 11	0	76	48	62	99
24	95	76	85.5	2 NE., 3 E., 9 NE	4 to 13	0	60	39	49	94
25	96	76	86.0	1 NE., 6 E., 1 NE., 6 E	5 to 17	0	60	31	45	88
26	88	72	80.0	9 E., 1 SE., 2 E., 2 SE	4 to 17	.19	98	85	91	48

RELATION OF SEX TO DISPERSION

Unfortunately the percentage of males and females among the flies liberated was not determined, hence no information can be gained from this test as to the relative proportion of the sexes among the flies recovered. It will be noted (Table II) that in the first ring of traps about the point of liberation 88 per cent of the marked house flies taken were females and 63.2 per cent of the screw-worm flies were females. In the second ring of traps the percentages of females of these two species were 92.8 per cent and 70.7 per cent, respectively; in the third ring 92.8 per cent and 78.3 per cent, and in the fourth ring 90.4 per cent and 75.5 per cent. So few marked flies of the other species were recovered that the sex data on them will not be discussed separately. It is noted that among the 4,652 marked flies recovered 3,952 were females, or 84.9 per cent. It might be supposed that if dissemination is induced largely by instinctive search for food and places to oviposit the females might tend to travel farther than the males, but the foregoing figures hardly justify

this conclusion, especially in the case of the house fly, which showed a fairly uniform relation between the number of males and females caught in the different circles of traps. Among the screw-worm flies the females appear to have shown a slightly more marked tendency toward distant dispersion.

RAPIDITY OF DISPERSION

In this test it was not possible to get very accurate data on the rapidity with which the different traps were reached, owing to the fact that the traps were not examined at frequent enough intervals to get accurate figures. A number of specimens of house flies were recovered in every trap, including the most remote ones, the day following liberation when the first examination was made, and the vast majority of all marked flies recovered had entered the traps at the end of the fourth day. Several specimens were recovered in nearly all of the traps up to the eighth day, and a few specimens were recovered after this, the last being taken on the twelfth day. It is possible that some may have been recovered after this, but as a second liberation was made on that date it would be impossible to determine whether the flies belonged to the first or second lot. Computation of the percentage of the flies recovered on different days in the different circles of traps indicates that the marked flies reached the nearer traps somewhat sooner than the more distant ones.

SECOND DISPERSION TEST AT DALLAS, TEX., 1916

On July 11, 1916, at 12.45 p. m., about 8,000 flies, marked with red chalk, were liberated at the same point as in the previous experiments. These consisted of about 70 per cent *Musca domestica*, 25 per cent *Chrysomya macellaria*, 4 per cent *Phormia regina*, 1 per cent *Lucilia sericata*, *Ophyra* spp. and *Sarcophaga* spp., etc. On July 12, during the forenoon, a second liberation of about 25,000 marked flies was made. This lot was estimated at about 50 per cent *M. domestica*, 40 per cent *C. macellaria*, 9 per cent *P. regina*, and 1 per cent *L. sericata*, *Sarcophaga* spp., etc. Part of these flies were colored with paint pigment known as "fast red" and others with powdered chalk. At the time of release a 7- or 8-mile breeze was blowing from the south, the sky was clear, and the sun was very hot.

In this test the traps in the first and second circles of the previous experiment were moved beyond the outer circle in the previous experiment, the traps in circles 3 and 4 in the previous experiment becoming numbers 1 and 2 in the present test. The environment of these eight traps has been mentioned in the discussion of the previous experiment. The positions of the outer rings of traps are briefly described as follows: West, No. 3, by farm lot in trees, stable and house 50 yards distant; No. 4, by grocery store in edge of city of Dallas. North, No. 3, in edge of cornfield ⅓ mile north of the village of Reinhardt; No. 4, in cornfield

1/8 mile from nearest farmhouse. East, No. 3, in weeds 200 yards east of a farmhouse; No. 4, 100 yards from farmhouse and pens where much live stock is kept. South, No. 3, under tree by small grocery store at cross-roads; No. 4, in village of Elam near stores and house. The distances from the release point to the traps are given in Table IV.

TABLE IV.—*Second dispersion test at Dallas, Tex. Species and sex of marked flies recovered in rings of traps about point of liberation*

Trap No.	Distance from place of release.	*Musca domestica.*		*Chrysomya macellaria.*		*Phormia regina.*		*Ophyra leucostoma.*		*Sarcophaga.* spp.		Undetermined.		Total.		Grand total.
	Miles.	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
1 W	1.6	0	33	10	28	0	5	0	1	0	0	0	0	10	67	77
1 N	1.8	0	8	3	18	0	0	0	0	0	0	0	0	3	26	29
1 E	2.0	0	10	8	19	0	2	0	0	0	0	0	0	8	31	39
1 S	2.0	0	4	1	3	0	0	0	1	0	0	0	0	1	8	9
Total		0	55	22	68	0	7	0	2	0	0	0	0	22	132	154
2 W	2.5	0	23	8	18	0	2	0	0	0	0	0	1	8	44	52
2 N	2.4	0	29	9	13	0	0	0	1	0	1	0	0	9	44	53
2 E	3.5	0	8	1	5	0	0	0	0	0	0	0	0	1	13	14
2 S	2.9	0	1	1	0	0	1	0	0	0	0	0	0	1	2	3
Total		0	61	19	36	0	3	0	1	0	1	0	1	19	103	122
3 W	3.0	0	5	6	25	2	10	0	2	0	1	0	0	8	43	51
3 N	3.4	0	4	4	18	0	2	0	[a]1	0	0	0	0	4	25	29
3 E	4.2	0	1	1	4	0	0	0	0	0	0	0	0	1	5	6
3 S	4.0	0	3	2	6	0	0	0	0	0	0	0	0	2	9	11
Total		0	13	13	53	2	12	0	3	0	1	0	0	15	82	97
4 W	3.75	0	6	0	9	0	1	0	1	0	0	0	0	0	17	17
4 N	4.4	0	9	11	35	0	0	0	0	0	0	0	0	11	44	55
4 E	5.0	0	2	3	5	0	0	0	0	0	0	0	0	3	7	10
4 S	5.0	0	0	0	2	0	1	0	0	0	0	0	0	0	3	3
Total		0	17	14	51	0	2	0	1	0	0	0	0	14	71	85
Grand total		0	146	68	208	2	24	0	7	0	2	0	1	70	388	458

[a] *Ophyra aenescens* Wied.

Since a single fly liberated at the beginning of the first test was recovered on July 11, it is barely possible that other marked specimens liberated at that time were recovered after July 12. In this discussion they would be considered as liberated in the second series.

In this experiment the number of colored flies captured was very much less than the number caught in the first test beginning June 30. The total number of marked flies recovered in the different directions from the point of liberation was 458, as compared with 4,652 in the first test. It is true that almost one-third more flies were liberated in the first test than in this one, but the proportion of those recovered is much smaller. It is also true that two of the rings of traps were much more distant from the point of liberation than in the first test; however, when we compare the catches in the two inner circles of traps in this test with the two outer ones in the first—positions which were the same in the two experiments—

we find that the percentage of marked flies recovered as compared with those liberated was 0.08 and 0.16, respectively (Tables II and IV).

Another factor which should have reduced the proportion of the marked flies recovered in these two rings of traps in the first test was that many were captured in the two circles of traps nearer the point of release. This difference in the number of marked flies recovered in the two experiments seems to be chargeable logically to climatic conditions. On July 11 and the following days the temperature ran markedly higher than during the period immediately after the first liberation, and the humidity ran correspondingly lower. This would have the effect of reducing the longevity, and possibly causing flies to seek shelter and moisture rather than to disperse freely.

The proportions of the several species among the marked flies released (estimated) and recovered, respectively, were as follows: *Musca domestica*, 52 per cent and 32 per cent; *Chrysomya macellaria*, 36 per cent and 60 per cent; *Phormia regina*, 8 per cent and 6 per cent; other species 0.01 per cent and 1.53 per cent. The hot weather was probably a greater inhibiting factor in the case of the house fly and *P. regina* than in that of *C. macellaria*. The optimum temperature is higher in the case of the last species. The make-up of the total catch in the recovery traps was not determined, but it was evident that there was a marked decrease in the total catch toward the end of the period and a marked reduction in the number of *P. regina* captured.

DISTANCE OF DISPERSION

Numerous marked specimens of *Musca domestica* and *Chrysomya macellaria* reached the more distant ring of traps, several specimens of both species being recovered in trap No. 4 east, 5 miles from the point of liberation, and three specimens of *C. macellaria* in No. 4, 5 miles south. Not a single *M. domestica* was recovered in No. 4 south, however. *Phormia regina* also reached the outside ring of traps, one specimen being taken in No. 4 south (5 miles), one in No. 4 west (3.75 miles), and many in No. 3 west (3 miles). One specimen of *Ophyra leucostoma* Wied. was recovered in No. 4 west (3.75 miles) and one specimen of *O. aenescens* Wied. in No. 3 north (3.4 miles). Only two specimens of *Sarcophaga* spp. were recovered, one in No. 3 west (3 miles) and one in No. 2 north (2.4 miles). The number of flies recovered at different distances is shown in detail in Table IV.

DIRECTION OF SPREAD

The individual species should be treated separately, but the several species recovered in the different directions seem to remain in about the same proportion with the exception of *Phormia regina*, which showed a marked tendency to go westward, 20 specimens (77 per cent) being taken in the west line of traps, while only two were taken in the traps in

each of the other directions. The large number of marked flies taken in the lines of traps west and north as compared with those taken in the traps east and south strongly indicates that there was a tendency in this experiment also to travel with the wind, as the prevailing wind during the first nine days was from the south to east, and during the first five days the wind was almost constantly from the south and southeast. The respective number and percentage of house flies and screw-worm flies recovered in the different directions were as follows (based on total number of marked specimens of each species): West, 67 (45.89 per cent) and 104 (37.68 per cent); north, 50 (34.25 per cent) and 111 (40.22 per cent); east, 21 (14.38 per cent) and 46 (16.67 per cent); south, 8 (5.48 per cent) and 15 (5.43 per cent). All but one of the specimens of Ophyra and Sarcophaga were recovered in the west and north traps.

The influence of traffic along the roads on dispersion would be practically the same as in the first test; that is, the principal traffic was east and west of the point of liberation. Heavy traffic also passed between No. 3 and 4 north and 3 and 4 south.

SEX IN RELATION TO MIGRATORY TENDENCY

In this test it is noteworthy that not a single male house fly was recovered. With the screw-worm fly the percentage of females recovered was 75.3. This percentage was remarkably uniform in the different directions from the point of liberation, ranging from 71.7 on the east line of traps to 76.9 on the west line. Considering the distance of dispersion of the males and females of the screw worm there is a slight indication that there was a greater tendency toward wide dispersion among the females than among the males. It may be worthy of note that the females appeared to enter the various traps in greater numbers during the early part of the recovery period than did the males. For instance, the percentage of males entering all of the traps during the first three days of the test was about 21, while during the remainder of the recovery period it was 45. This should not be construed as meaning that the males were necessarily slower in dispersion, but they may have displayed less eagerness to find food and hence to become entrapped.

RAPIDITY OF DISPERSION

Comparatively few marked flies were recovered in the traps when emptied the first day after liberation. This was especially notable in the fourth ring of traps (3.75 to 5 miles from the point of liberation) where only a single specimen of *Chrysomya macellaria* was recovered. The recoveries the second day (July 13) were markedly larger than the first, this day giving the maximum daily catch (134) of *C. macellaria* for the period. This species seemed on this day to be generally distributed throughout the area, nearly all traps showing a marked decline in the

number captured after this date. The house flies appear to have become widely disseminated by the end of the second day, and the maximum number was recovered on the fourth day (July 15). This maximum, however, was not attained through large numbers being captured in the more distant traps but by an increased catch in the two inner rings. The trapping was continued until July 26, the last *C. macellaria* being captured on the eleventh day (July 22), one specimen appearing on that date in each of traps No. 4 north and No. 4 south. The last house flies recovered were taken on the same date, one each in No. 1 south and No. 3 south. In No. 2 north 7 house flies were recovered on July 21, a much larger proportion on this late date than was observed in any other trap. The total in this trap was 29.

Several *Phormia regina* out of a total of 26 recovered were taken soon after liberation, one appearing in No. 2 west and three in No. 3 west on the first day. The last *P. regina* was recovered on the tenth day (July 21) in No. 3 west.

There were too few specimens of Ophyra liberated or recovered to draw conclusions, but it may be stated that the first specimens recovered were taken in No. 3 west, No. 2 north, and No. 1 south (one in each) on the third day after liberation and the last in No. 2 west on the seventh day (July 18).

THIRD DISPERSION TEST AT DALLAS, TEX.

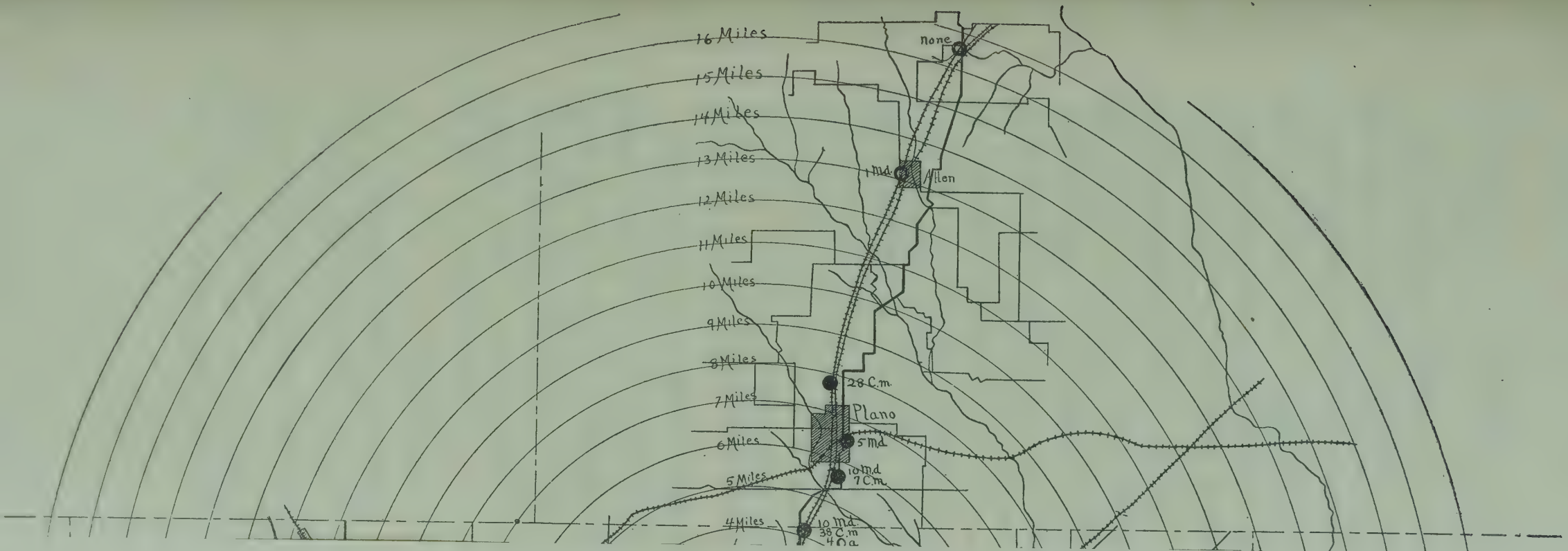
The evidence secured in the second test very clearly indicated that the maximum distance of normal dispersion of the various species of flies was far greater than the outer circle of traps in that test, which was approximately 5 miles. Accordingly plans were laid for another test to determine if possible the maximum distance of dissemination. For this test the region lying to the north of Dallas was chosen. The point of liberation selected was approximately 7 miles from the edge of the city of Dallas on the main north and south thoroughfare known as "Kings Highway." This region is highly developed agriculturally, being thickly dotted in all directions with farms, most of which maintain some live stock. The contour of the country is not markedly different from that where the first and second tests east of Dallas were conducted. Within the area under consideration are a number of creeks bordered with woods of greater or less width. For the most part, however, the country is open cultivated land more or less closely covered with a network of roads, several steam railways, and one electric line. As has been stated, the point of liberation was on the most heavily traveled highway running north from Dallas. On this road, about 4 miles south of the point of liberation, is the town of Vickery (about 100 population) and to the north 1 mile the town of Richardson (500 population), Plano (1,700 population) 6½ miles, and Allen (300 population) 13 miles. To the east are a number

of small community centers within a radius of 4 miles from the point of liberation and the towns of Garland (1,400 population) 6 miles, Rowlett (150 population) 10 miles, and Rockwall (1,400 population) 16 miles. To the west are also several community centers and the towns of Farmers Branch (250 population) 9½ miles and Carrollton (575 population) 10 miles.

Fairly direct though not heavily traveled roads lead east and west from near the point of liberation. The traffic on the east road outward from a corner 5½ miles east of the point of release is heavy, being probably equal to that north and south of the point of release. The traps were set along these highways where they could be examined and rebaited expeditiously.

The principal roads, location of towns and main streams, and their relation to the point of release and the recovery traps are indicated on the accompanying map (fig. 2). It will be noted that the north line of traps bears somewhat to the east of due north from the point of release and those to the south are about 2 miles west of south, while the east traps were about 1 mile south of east and those to the west practically due west. Sixteen recovery traps were utilized, four being set in each direction from the release point. These were 18-inch cone traps. The immediate environment of the traps may be described briefly as follows: West, No. 1, 4½ miles, under shed in cotton field, no stock near except chickens; No. 2, 6 miles, adjacent to hogpen and near mule barn on farm; No. 3, 7 miles, under shed by mule barn, cow lot, and hogpen; No. 4, 8.1 miles, by crib next to hogpen and cow lot. North, No. 1, 4.1 miles, under hedge near hogpen; No. 2, 5.6 miles, in old hogpen near barn, mules and chickens kept; No. 3, 6.3 miles, under seed house at gin in edge of town; No. 4, 7.8 miles, under hedge in cotton field, no stock on farm. East, No. 1, 4.7 miles, under empty corn crib, no stock near; No. 2, 6.2 miles, under water tank by horse barn; No. 3, 7.2 miles, adjacent to hogpen and mule barn; No. 4, 8 miles, in vacant shed one-third mile from main road and 100 yards from stock barns. South, No. 1, 4.6 miles, near house in edge of village, no live stock near; No. 2, 6.1 miles, adjacent to house, barns, and hogpen; No. 3, 7.3 miles, in shed near yards with all classes of live stock; No. 4, 8.2 miles, under tree in edge of city.

On September 17, the day prior to liberation, all traps were baited with fresh gut slime, and they were all rebaited on September 23 and 27 and October 3. Beginning with September 20, and continuing until September 28, the flies were killed and examined daily in all traps. The traps were not emptied on September 29, October 1, 2, and 4 (except No. 1 and 2 south). October 5 was the last date when all traps were emptied and the last date upon which any marked flies were recovered. On October 7 all flies in traps 2 and 3 south, and on October 8 all flies in traps 4 west and 4 south were killed and examined, but no marked flies were found.


16 Miles
15 Miles
14 Miles
13 Miles
12 Miles
11 Miles
10 Miles
9 Miles
8 Miles
7 Miles
6 Miles
5 Miles
4 Miles
none
1 md.
Allen
28 C.m.
Plano
5 md.
10 md.
7 C.m.
10 md.
38 C.m
40 a

On September 18, between 10 and 11 a. m., about 20,000 marked flies were liberated, and between 3 and 4 p. m., 40,000 were released. These flies were secured by means of traps at an abattoir in Dallas, the first lot being taken between noon of the previous day and 9 o'clock on the morning of release and the second lot between 9 a. m. and 2 p. m. on the same date. The flies were transported to the point of release in screened cylinders containing green branches. They were then well colored with powdered red crayon by placing the cylinders in a canvas bag. When liberated the flies appeared to be very hungry, and many of them clung to our clothing and bodies in an effort to secure moisture, but the vast majority flew into the air, some apparently going up 20 or 30 feet and disappearing in all directions, but seemingly more going northward than in other directions. At the time of release the sky was clear and a gentle breeze was blowing from the south. The maximum temperature for the day was 95° F. All flies were brushed off clothing and cylinders, and two stops were made after the scene of release was left in order to make sure that no colored flies were following.

The proportions of the different species released were about as follows: *Musca domestica* 42.32 per cent, *Chrysomya macellaria* 54.90 per cent, *Phormia regina* 1.25 per cent, *Sarcophaga* spp. 1.25 per cent, *Lucilia sericata* 0.14 per cent, *Ophyra leucostoma* and *O. aenescens* 0.14 per cent. The approximate averages of the species in the entire catch in the 16 traps were: *Musca domestica* 44 per cent, *C. macellaria* 44 per cent, *P. regina* 1 per cent, *Ophyra* spp. 0.06 per cent, *Lucilia* spp. 1 per cent, *Sarcophaga* spp. 8 per cent.

The proportion of the species among the marked specimens recovered was *Musca domestica* 20 per cent, *Chrysomya macellaria* 78.84 per cent, *Ophyra aenescens* 1.16 per cent. No marked specimens of *Phormia regina*, *Lucilia sericata*, or *Sarcophaga* spp. were captured. Comparing these percentages with the percentages of the species released, we note that the percentage of Ophyra recovered is over eight times, *C. macellaria* slightly less than one and one-half times, and *M. domestica* less than one-half the percentage released.

DISTANCE OF DISPERSION

As is shown in Table V, the number of marked house flies reaching the four concentric rings of traps from the center outward was 30, 23, 11, and 5, a total of 69. Expressing this in percentages, we have 43.5, 33.3, 15.9, and 7.3. The maximum distance recorded for this species in this test was 8.1 miles in trap No. 4 west, where three marked flies were recovered. This was almost equaled in trap No. 4 east (7.9 miles) where two colored house flies were recovered.

TABLE V.—*Third dispersion test at Dallas, Tex. Species and sex of marked flies recovered in rings of traps about point of liberation*

Trap No.	Distance from place of release.	*Musca domestica.*				*Chrysomya macellaria.*				*Ophyra aenescens.*				Total.				Grand total (number).
		Number.		Per cent.		Number.		Per cent.		Number.		Per cent.		Number.		Per cent.		
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
	Miles.																	
1 W....	4.5	1	2	33.3	66.7	5	43	10.4	89.6	0	0	0	0	6	45	11.8	88.2	51
1 N.....	4.1	0	15	00.0	100.0	6	32	16.8	84.2	2	2	50	50	8	49	14.04	85.96	57
1 E.....	4.7	0	2	00.0	100.0	8	23	25.8	74.2	0	0	0	0	8	25	24.2	75.8	33
1 S.....	4.6	1	9	10.0	90.0	4	34	10.5	89.5	0	0	0	0	5	43	10.4	89.6	48
Total...		2	28	6.67	93.33	23	132	14.8	85.2	2	2	50	50	27	162	14.29	85.71	189
2 W....	6.0	0	1	.0	100.0	1	20	4.8	95.2	0	0	....	....	1	21	4.5	95.5	22
2 N.....	5.6	3	10	23.08	76.92	0	7	.0	100.0	0	0	....	....	3	17	15.0	85.0	20
2 E.....	6.2	0	0	.....	.....	0	13	.0	100.0	0	0	....	....	0	13	.0	100.0	13
2 S.....	6.1	1	8	11.1	88.9	3	10	23.1	76.9	0	0	....	....	4	18	18.2	81.8	22
Total...		4	19	17.39	82.61	4	50	7.4	92.6	0	0	....	....	8	69	10.39	89.61	77
3 W....	7.0	0	2	.0	100.0	0	4	.0	100.0	0	0	....	....	0	6	.0	100.00	6
3 N.....	6.3	2	3	40.0	60.0	0	0	.....	.....	0	0	....	....	2	3	40.0	60.0	5
3 E.....	7.2	1	3	25.0	75.0	0	6	.0	100.0	0	0	....	....	1	9	10.0	90.0	10
3 S.....	7.3	0	0	.....	.....	0	3	.0	100.0	0	0	....	....	0	3	.0	100.0	3
Total...		3	8	27.3	72.7	0	13	.0	100.0	0	0	....	....	3	21	12.5	87.5	24
4 W....	8.1	0	3	.0	100.0	0	8	.0	100.0	0	0	....	....	0	11	.0	100.0	11
4 N.....	7.8	0	0	.....	.....	3	25	10.7	89.3	0	0	....	....	3	25	10.7	89.3	28
4 E.....	8.0	0	2	.0	100.0	0	5	0.0	100.0	0	0	....	....	0	7	.0	100.0	7
4 S.....	8.2	0	0	.....	.....	2	7	22.2	77.8	0	0	....	....	2	7	22.2	77.8	9
Total...		0	5	.0	100.0	5	45	10.0	90.0	0	0	....	....	5	50	9.1	90.9	55
Grand total..		9	60	13.04	86.96	32	240	11.76	88.24	2	2	50	50	43	302	12.44	87.56	345

As will be noted, a considerably larger number of marked *Chrysomya macellaria* were recovered, the total being 272. Of these, 155 (57 per cent) were taken in the first ring of traps, 54 (19.8 per cent) in the second ring, 13 (4.8 per cent) in the third ring, and 50 (18.4 per cent) in the fourth ring. It will be noted that a considerable number of marked flies of this species were taken in each of the traps in the outer ring, which is approximately 8 miles from the point of liberation, the maximum distance being 8.2 miles in No. 4 south, where 9 colored flies were recovered. The comparatively small number of marked screw-worm flies taken in the third ring of traps is noteworthy, but this can be explained largely on the basis of the comparatively unfavorable location of these traps for the collection of this species. This matter will be discussed subsequently.

The only other species of marked flies captured was *Ophyra aenescens*, four specimens of which were taken in No. 1 north, 4.4 miles from the point of liberation.

TABLE VI.—*Third dispersion test at Dallas, Tex. Species and sex of marked flies recovered in different directions from point of liberation*

Trap No.	Distance from place of release.	*Musca domestica.*				*Chrysomya macellaria.*				*Ophyra aenescens.*				Total.				Grand total (number).
		Number.		Per cent.		Number.		Per cent.		Number.		Per cent.		Number.		Per cent.		
	Miles.	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
1 W....	4.5	1	2	33	67.0	5	43	10.4	89.6	0	0	....	....	6	45	11.8	88.2	51
2 W....	6.0	0	1	00	100.0	1	20	4.8	95.2	0	0	....	....	1	21	4.5	95.5	22
3 W....	7.0	0	2	00	100.0	0	4	0	100.0	0	0	....	....	0	6	0	100.0	6
4 W....	8.1	0	3	00	100.0	0	8	0	100.0	0	0	....	....	0	11	0	100.0	11
Total...		1	8	11.2	88.8	6	75	7.4	92.6	0	0	....	....	7	83	7.8	92.2	90
1 N....	4.1	0	15	00	100.0	6	32	15.8	84.2	2	2	50	50	8	49	14.04	85.96	57
2 N....	5.6	3	10	23	77.0	0	7	0	100.0	0	0	....	....	3	17	15.0	85.0	20
3 N....	6.3	2	3	40	60.0	0	0	.....	.....	0	0	....	....	2	3	40.0	60.0	5
4 N....	7.8	0	0	.....	.....	3	25	10.7	89.3	0	0	....	....	3	25	10.7	89.3	28
Total...		5	28	15.15	84.85	9	64	12.3	87.7	2	2	50	50	16	94	14.55	85.45	110
1 E.....	4.7	0	2	00	100.0	8	23	25.9	74.1	0	0	....	....	8	25	24.2	75.8	33
2 E.....	6.2	0	0	.....	.....	0	13	0	100.0	0	0	....	....	0	13	0	100.0	13
3 E.....	7.2	1	3	25	75.0	0	6	0	100.0	0	0	....	....	1	9	10.0	90.0	10
4 E.....	8.0	0	2	00	100.0	0	5	0	100.0	0	0	....	....	0	7	0	100.0	7
Total...		1	7	12.5	87.5	8	47	14.5	85.5	0	0	....	....	9	54	14.3	85.7	63
1 S.....	4.6	1	9	10.0	90.0	4	34	10.6	89.4	0	0	....	....	5	43	10.4	89.6	48
2 S.....	6.1	1	8	11.2	88.8	3	10	23.1	76.9	0	0	....	....	4	18	18.2	81.8	22
3 S.....	7.3	0	0	.....	.....	0	3	0	100.0	0	0	....	....	0	3	0	100.0	3
4 S.....	8.2	0	0	.....	.....	2	7	22.3	77.7	0	0	....	....	2	7	22.3	77.7	9
Total...		2	17	10.6	89.4	9	54	14.3	85.7	0	0	....	....	11	71	13.4	86.6	82
Grand total..		9	60	13.04	86.96	32	240	11.76	88.24	2	2	50	50	43	302	12.44	87.56	345

DIRECTION OF FLIGHT

In Table VI the numbers of marked flies recovered in the different directions from the point of liberation are set forth. In considering the house fly from the standpoint of the direction of travel as indicated by the number of marked specimens recovered, probably the most striking fact is that 33, or 47.8 per cent, of the marked flies were taken in the four traps to the north of the point of release, and the next largest number—19, or 27.5 per cent—were recovered in the traps in the opposite direction. The catches to the west and east were almost identical, the west with 9 flies, or 13 per cent, and the east with 8 flies, or 11.6 per cent.

In considering the factors which might influence dissemination of the house fly we immediately think of the attraction of feeding and breeding places, the character and amount of travel along the highways, and the direction and velocity of the wind. The attraction of odors from towns and cities would tend to favor the migration to the north and south, since there are several towns located along the north line of traps and a few to the south and east. If the flies are inclined to go toward odors borne a considerable distance on the wind, one would expect a greater number to the south, since the volume of odors from the city of Dallas

would be stronger than that originating elsewhere. If the attraction exerted is of more limited range, so that the proximity of farm buildings and small towns would exert the greater influence, we would probably find the greater number of specimens to the north, east, and south, which agrees fairly well with the facts.

TABLE VII.—*Climatological data relating to third dispersion test at Dallas, Tex.*

Date.	Temperature.			Wind.		Rain.	Humidity.			Actual sun.
	Max.	Min.	Mean.	Direction during successive hours from 6 a. m. to 8 p. m.	Velocity during day.		Max.	Min.	Mean.	
1918.	°F.	°F.	°F.		Miles.	Inch.	P. ct.	P. ct.	P. ct.	P. ct.
Sept. 18...	91	66	78.5	2 E., 2 SE., 8 S., 2 SE.....	5 to 10	0	78	36	57	80
19...	79	62	70.5	1 S., 5 NE., 6 E., 2 NW.....	5 to 21	0.57	65	56	60	34
20...	68	55	61.5	14 N.....	7 to 13	0	72	55	63	70
21...	70	47	58.5	2 N., 6 E., 2 NE., 4 E.....	1 to 9	0	83	34	58	100
22...	76	51	63.5	2 E., 11 SE., 1 S.....	6 to 9	0	67	33	50	99
23...	80	53	66.5	2 E., 12 SE.....	4 to 12	0	61	25	43	99
24...	85	55	70.0	6 SE., 1 S., 4 SE., 3 S.....	7 to 17	0	68	45	56	93
25...	87	60	73.5	5 SE., 6 S., 3 SE.....	10 to 20	0	85	52	68	78
26...	72	63	67.5	3 E., 3 N., 1 NE., 7 NW.....	4 to 11	.86	83	69	76	25
27...	72	55	63.5	4 N., 1 NW., 9 N.....	4 to 14	0	78	42	60	75
28...	75	48	61.5	12 NW., 1 W., 1 NW.....	4 to 9	0	87	35	61	100
29...	85	51	68.0	6 W., 3 NW., 2 NE., 3 E....	2 to 7	0	73	29	51	99
30...	88	60	74.0	5 W., 1 SW., 4 S., 4 E.....	3 to 7	0	61	23	42	100
Oct. 1...	89	62	75.5	4 E., 7 S., 3 SE.....	1 to 10	0	62	27	44	93
2...	87	61	74.0	3 SE., 4 W., 5 SE., 2 E.....	2 to 7	0	66	33	49	93
3...	89	63	76.0	3 E., 1 S., 3 SE., 7 S.....	2 to 7	0	61	29	45	98
4...	89	66	77.5	2 E., 12 SE.....	5 to 9	0	58	32	45	100
5...	89	67	78.0	3 S., 2 SW., 7 S., 2 SE.....	3 to 13	0	70	38	54	62

As will be seen by referring to Table VII, the period during which this test was conducted was extremely unfavorable for determining the possible influence of wind on the dissemination of flies. During the entire period of recovery there was no considerable number of consecutive days in which the wind was constant. In other words, the wind condition would be expressed as "choppy," and with its continual varying from one quarter to another it is almost impossible for one to weigh the wind influence on dissemination in this test. For 7 hours following the liberation of the first lot of flies there was blowing a 5- to 10-mile breeze from the south. It is conceivable that the flies when liberated drifted rapidly northward with the wind and that many were subsequently carried back and in other directions with the wind changes. The assumption that they traveled with the wind is hardly borne out by the recovery of several flies in the south traps on the next day (Table VIII) and by the recovery of four flies in the east traps on September 22, where they must have gone at right angles to or against the wind. Moreover, there is strong evidence, as indicated by the collections of marked house flies in the four traps to the west of the point of liberation, that there is no pronounced tendency for them to travel with the wind, or at least that tendency is easily overcome by other influencing factors. During the first 5 days of the test not a single marked house fly was recovered in the west traps, and during this period the prevailing wind during the hours

of fly activity was from the east and southeast, 44 hours of wind being from those directions while the wind came from other directions for 37 hours, 16 of these being from the north and 7 from the northeast while no west or southwest wind whatever was experienced. The fact that all the flies captured in the west line of traps were taken on two days, September 24 and 25, 5 on the former and 4 on the latter, is peculiar, but no explanation is apparent. The prevailing wind on these two days was from the southeast and south and on the two days preceding from the southeast, while during the days preceding September 22 and succeeding September 25 no southwest wind was experienced during the period when marked house flies were being recovered.

The question of the amount of traffic along the highways was mentioned in the introductory remarks on this flight experiment. It is certainly true that there is far more traffic along the highway running north and south from the point of liberation than to the east and west, and this corresponds with the greatest dissemination. There is, however, heavier traffic to the south of the point than there is to the north, but many less flies were recovered. There is a comparatively small amount of traffic which would induce house flies to congregate and follow loads over any of the roads. No doubt the most attractive carriers would be milk wagons going into Dallas and returning to the rural districts, and these are not very numerous on the highways to the north. Other slow-moving vehicles would consist mostly of loads of cottonseed hulls and meal, which are not attractive, and miscellaneous groceries and supplies which would not be considered especially attractive. No garbage or manure is hauled over the roads in any direction. The major part of the traffic consists of passenger-carrying automobiles and some motor trucks. At the time this test was being carried on there were many families with various kinds of household goods and food driving teams north and south on the main highway. These slow-moving covered wagons might readily serve as a means of transport for house flies for some distance.

The dissemination of *Chrysomya macellaria* in the different directions was much more uniform than that of the house fly. Of the total of 272 marked screw-worm flies recovered, 81, or 29.8 per cent, were recovered in the four traps to the west, 73, or 26.8 per cent, to the north, 55, or 20.2 per cent to the east, and 63, or 23.2 per cent, to the south. The screw-worm fly, which is not especially attracted by urban conditions or habitations and is not inclined to come to or be carried by vehicles unless loaded with meat or meat products, would not be expected to show the same tendencies in dissemination as the house fly, and this seems to be indicated by the number of marked flies recovered in the different directions. With choppy wind conditions it would be equally as hard to determine the wind influence on this species as on the house fly.

TABLE VIII.—*Third dispersion test at Dallas, Tex. Number of marked Musca domestica and Chrysomya macellaria captured on successive dates after liberation*

Trap No.	Sept. 19.		Sept. 20.		Sept. 21.		Sept. 22.		Sept. 23.		Sept. 24.		Sept. 25.		Sept 26.		Sept. 27.		Sept. 28.		Sept. 30.		Oct. 3.		Oct. 5.		Total.	
	M. d.	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*
1 W	0	39	0	2	0	0	0	4	0	1	2	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	3	48
2 W	0	13	0	0	0	0	0	1	0	1	0	3	1	2	0	0	0	0	0	0	0	0	0	0	0	1	1	21
3 W	0	3	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	4
4 W	0	4	0	1	0	0	0	0	0	0	1	2	2	0	0	0	0	0	0	0	0	0	0	1	0	0	3	8
Total	0	59	0	3	0	0	0	5	0	2	5	5	4	3	0	1	0	0	0	1	0	0	0	1	0	1	9	81
1 N	5	25	1	4	1	0	0	2	0	0	3	6	4	0	1	1	0	0	0	0	0	0	0	0	0	0	15	38
2 N	3	4	2	0	2	0	0	1	2	1	0	1	2	0	2	0	0	0	0	0	0	0	0	0	0	0	13	7
3 N	1	0	1	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0
4 N	0	15	0	2	0	2	0	5	0	2	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	28
Total	9	44	4	6	3	2	0	8	3	3	5	8	6	0	3	1	0	0	0	0	0	1	0	0	0	0	33	73
1 E	1	22	1	1	0	0	0	1	0	0	0	6	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	31
2 E	0	9	0	1	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
3 E	0	4	0	1	0	0	3	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	6
4 E	0	4	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	5
Total	1	39	1	4	0	0	4	3	0	0	1	8	1	1	0	0	0	0	0	0	0	0	0	0	0	0	8	55
1 S	3	16	0	5	1	1	0	8	4	5	1	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	9	38
2 S	2	5	1	4	0	0	1	1	1	0	2	1	1	0	0	1	1	0	0	0	0	0	1	0	0	1	10	13
3 S	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
4 S	0	2	0	2	0	0	0	1	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	9
Total	5	23	1	11	1	1	1	10	5	11	3	1	1	2	0	3	1	0	0	0	0	0	1	0	0	1	19	63
Grand total	15	165	6	24	4	3	5	26	8	16	14	22	12	6	3	5	1	0	0	1	0	1	1	1	0	2	69	272
Percentage [a]	21. 74	.....	8. 69	.....	5. 80	.....	7. 25	.....	11. 59	.....	20. 29	.....	17. 39	.....	4. 35	.....	1. 45	.....	0	.....	0	.....	1. 45	.....	0	.....	100	.....
Percentage [b]	.....	60. 65	.....	8. 82	.....	1. 10	.....	9. 56	.....	5. 88	.....	8. 09	.....	2. 21	.....	1. 84	.....	0	.....	0. 37	.....	. 37	.....	. 37	.....	. 74	.....	100

[a] Percentage of marked *Musca domestica* caught each day based on total marked *M. domestica* recovered.
[b] Percentage of marked *Chrysomya macellaria* caught each day based on total marked *C. macellaria* recovered.

It is peculiar that all four of the specimens of *Ophyra aenescens* were taken in a single trap, No. 1 north. Little is known of the habits and responses to various stimuli of this species, but we usually think of it as being most abundant under rural conditions and in no sense a domestic species. These flies appear to be attracted to a considerable extent by carcasses in their last stages of decay and to some extent by hog manure.

RAPIDITY OF FLIGHT

Probably the best method of indicating the rapidity of dispersion from the point of release is by tabulating the number of marked specimens recovered in the various traps on succeeding days. These data are given in Table VIII. While it might appear that the house fly traveled very slowly to the west, none being recovered during the first 5 days, the fact that some marked specimens were taken in the most remote recovery trap in that direction on that date would indicate that this must be explained on some other basis than the time required to reach the traps. It will be noted that one marked specimen was taken in trap No. 3 north, 7.1 miles from the point of liberation, on the day following release, and two specimens were taken in trap No. 2 south, 6.1 miles, on the same day. The first specimen reaching No. 4 east, 7.9 miles, was on the fourth day after release. As no marked specimens were captured in traps No. 4 north and No. 3 and 4 south, any further statement as to the rapidity of dispersion to the outside of the 8-mile circle is hazardous. The fact that more marked flies were taken in the recovery traps on the first day than on any subsequent date after release indicates that spread of this species is rapid.

The large number of screw-worm flies recovered in all the traps on the first day after release indicates a very rapid and thorough dispersion of this species promptly after release. It is notable that more marked specimens were taken in the outside circle of traps, approximately 8 miles, on the first day following release than on any subsequent day. Of course the recoveries were affected markedly by temperatures as indicated by the comparatively few specimens taken on September 21 and 22 and the larger number taken September 24, which was a mild day. No doubt low temperatures would retard dissemination, but they would also have the effect of rendering the baits in the recovery traps less attractive and hence, even though the marked flies were in the vicinity, they might not be captured.

It would appear that in the case of neither the house fly nor the screw-worm fly were the recoveries any more gradual in the outer rings of traps than in the closer ones. In other words, there did not seem to be any percolation of the flies outward from the point of release. This, of course, would be influenced by the choppy wind condition already mentioned, provided the wind is a dominant factor in dispersion.

Three of the four specimens of *Ophyra aenescens* were taken on the third day following release and the other on the fourth day. This species, which is ordinarily thought of as having little power of flight, has shown rather remarkable powers of dissemination in this instance.

RELATION OF SEX TO DISPERSION

The proportion of the sexes of flies released, based on examination of a considerable number of specimens killed at the time of release, was: Males, *Musca domestica* 25.6 per cent, *Chrysomya macellaria* 15.6 per cent, *Phormia regina* none, *Lucilia sericata* none, *Ophyra* spp. none, *Sarcophaga* spp. 22.2 per cent. It will be noted by referring to Table V that the percentage of male *M. domestica* recovered (13) was not greatly different from the percentage (15.6) released. It is rather strange that one-half of the Ophyra recovered were males, while no males were noted in the material examined at the time of liberation.

Referring to *Musca domestica*, the proportion of males to females increased from the nearer circle of traps (6.7 per cent) outward to the third circle (27.3 per cent), but no males were caught in the outer circle. This does not indicate that this sex is more limited in distance of dispersion, since only 5 specimens were taken in the outer circle and the proportion of males to females was so small that the chance of recovering any among that number was meager. The maximum distance of flight of any male house fly was 7.2 miles, one specimen being recovered in No. 3 east. The proportion of sexes of this species in the different directions was about the same (Table VI), ranging from about 10.6 per cent males in the traps to the south to 15.2 per cent in the traps to the north.

In the case of *Chrysomya macellaria* the proportion of males to females varied considerably in the rings of traps at different distances, but not in a way to show any relation between sex and distance. The percentage of males in the first ring of traps (4.5 miles) was highest (14.8) and in the third ring lowest (none). The same erratic results are also apparent in the proportion of sexes in different directions, but the average percentage of males taken in the traps in the different directions is not very different, ranging from 7.4 in the west line of traps to 14.5 in the east line.

RELATION OF POSITION OF TRAPS TO THE RECOVERY OF MARKED FLIES

At the outset in these dispersion studies it became apparent that the environment of the recovery traps had a marked effect not only on the size of the catch but on the proportion of the species. In other words, certain situations occupied by recovery traps were preeminently house-fly situations while in others the blowflies dominated. As an illustration the results in the case of traps No. 3 and 4 north may be

cited. The first of these was in a village and most decidedly a house-fly environment, as indicated by a catch of 361 gm., of which 92 per cent were house flies. No. 4 was in a field removed from human habitations and hence more favorable for screw-worm flies. Here 78 per cent of the 548 gm. taken were screw-worm flies. Now note the number of marked flies recovered: Trap No. 3, 5 house flies and no screw-worm flies; trap No. 4, 28 screw-worm flies and no house flies.

It seems evident that those positions favorable for the capture of large numbers of one or more species of flies must be in the favorite haunts of

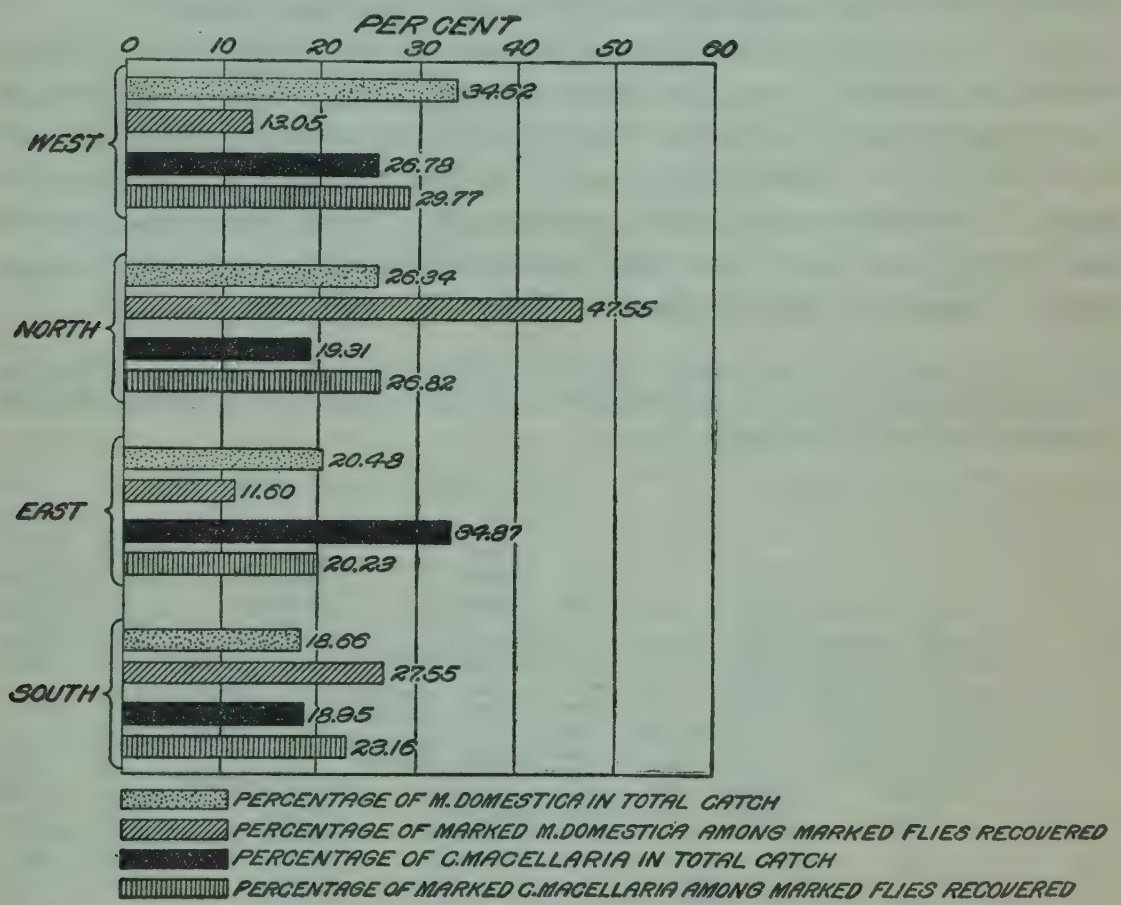


FIG. 3.—Diagram showing percentage of each species taken in each direction from point of liberation in the third dispersion test at Dallas, Tex. First, percentage of house flies in each direction as compared with total house flies caught; second, percentage of marked house flies as compared with the total marked house flies recovered; third, percentage of screw-worm flies in each direction as compared with total catch of this species; fourth, percentage of marked screw-worm flies as compared with the total marked screw-worm flies recovered.

the species so taken and that marked specimens in their movements would, like others, tend to congregate in such positions. Furthermore, it would appear that those traps so located as to catch the largest number of flies of a given species would have a better chance of capturing marked specimens. It is true that the occurrence of favorable breeding and feeding places in the immediate environs of a trap would favor large catches, but these very conditions would tend to assemble migrating marked individuals near the trap.

It was at first thought that by weighing the relative advantages of the positions of the traps in the capture of house flies and screw-worm flies

an index might be worked out and thus the difference in position equalized and the normal dispersion of the marked specimens be determined more accurately. This matter upon study became so complicated and so many unknown factors entered that it was dropped. It was finally concluded that a comparison of the relative positions might be made by computing in the form of percentage the relation between each of these principal species in the catches in the different traps and the total catch of each of the species in all the recovery traps. And again the percentage of marked flies of the two species in each trap as compared with the total marked flies of each species recovered in all traps was computed.

These two percentages—the percentage of total house flies and percentage of marked house flies taken in each trap—should approximate each other in size if all other factors such as distance, direction, etc., were eliminated. The same is true of the screw-worm fly. On the other hand, if the difference in these percentages is great, we must conclude that some other factor than mere local environment of the trap is dominant. These data are presented in Table IX and figure 3.

TABLE IX.—*Weight and proportion of catch of Musca domestica and Chrysomya macellaria compared with number and proportion of these species among marked specimens recovered in third test at Dallas, Tex.*

Trap No.	Total weight of *M. domestica* and *C. macellaria* caught.	Weight of *M. domestica* caught in trap.	Percentage of *M. domestica* compared with total in trap.	Weight of *C. macellaria* caught in trap.	Percentage of *C. macellaria* compared with total in trap.	Percentage of *M. domestica* in each trap compared with total *M. domestica* in all traps.	Number of marked *M. domestica* caught.	Percentage of marked *M. domestica* in each trap compared with total marked *M. domestica* in all traps.	Number of marked *C. macellaria* caught.	Percentage of *C. macellaria* in each trap compared with total *C. macellaria* in all traps.	Percentage of marked *C. macellaria* in each trap compared with total marked *C. macellaria* in all traps.
	Gm.	*Gm.*		*Gm.*							
1 west.....	368	109	29.6	259	70.4	3.29	3	4.35	48	7.84	17.64
2 west.....	674	262	38.9	412	61.1	7.90	1	1.45	21	12.47	7.72
3 west.....	242	181	74.8	61	25.2	5.46	2	2.90	4	1.84	1.47
4 west.....	752	596	79.2	156	20.8	17.97	3	4.35	8	4.72	2.94
1 north.....	316	179	56.6	137	43.4	5.40	15	21.74	38	4.15	13.97
2 north.....	283	238	84.1	45	15.9	7.18	13	18.84	7	1.36	2.57
3 north.....	361	333	92.2	28	7.8	10.04	5	7.24	0	.85	0.00
4 north.....	548	120	21.9	428	78.1	3.62	0	0.00	28	12.95	10.28
1 east.....	397	104	26.2	293	73.8	3.14	2	2.90	31	8.87	11.40
2 east.....	174	57	32.8	117	67.2	1.72	0	0.00	13	3.54	4.78
3 east.....	583	446	76.5	137	23.5	13.45	4	5.80	6	4.14	2.21
4 east.....	677	72	10.6	605	89.4	2.17	2	2.90	5	18.32	1.84
1 south.....	202	86	42.6	116	57.4	2.59	10	14.49	38	3.51	13.97
2 south.....	520	291	56.0	229	44.0	8.77	9	13.04	13	6.93	4.78
3 south.....	196	121	61.7	75	38.3	3.65	0	0.00	3	2.27	1.10
4 south.....	327	121	37.0	206	63.0	3.65	0	0.00	9	6.24	3.31
Total...	6,620	3,316	.....	3,304	.....	100.00	69	100.00	272	100.00	99.98

FOURTH DISPERSION TEST AT DALLAS, TEX.

Since marked specimens of both the house fly and the screw-worm fly were recovered to the maximum distance afforded by the recovery traps in the third dispersion test, it was decided to perform another experiment with the recovery stations at still greater distances from the point of liberation. The same point for liberating the flies was used in this test, but owing to difficulties encountered in collecting the flies and rebaiting the recovery traps to the north it was deemed advisable to set only two traps in that direction. The number of traps to the south was also decreased to two; the number east and west was increased to five in each direction, and one trap was placed in the southeast quadrant and one in the northeast quadrant in order to show more clearly whether or not the flies were following the main arteries of traffic which run approximately north and south and east and west.

The location of the traps in this test is indicated in figure 2. Their immediate environment and distance from the point of liberation were as follows: West, No. 1, 9.5 miles, in the edge of the town of Farmers Branch, some poultry and a cow on adjacent lot; No. 2, 10.9 miles, near farmhouse, hogpen, mule and cow lot adjacent; No. 3, 13.2 miles, under tool shed at a ranch headquarters, considerable live stock kept near by; No. 4, 15.8 miles, under sheep shed on farm, horses, mules, and hogs near; No. 5, 17.8 miles, against chicken house on farm, some hogs and mules 100 yards away. North, No. 1, 13.14 miles, in edge of town of Allen, in yard near hogpen; No. 2, 16.85 miles, in old barn on farm, hogs and mules in barnyard. East, No. 1, 9.3 miles, on farm, hogs, cows, and horses in barnyard; No. 2, 10.8 miles, under shed on farm, many pigs, sheep, and other animals in yard and small slaughterhouse 100 yards distant; No. 3, 13.1 miles, under water tank on farm, all classes of stock in adjacent yard; No. 4, 15.1 miles, under shed on farm, mules, hogs, and cattle near; No. 5, 17 miles, in shed adjacent to hogpen on farm, ½ mile beyond town of Rockwall. South, No. 1, 8.2 miles, under tree in edge of Dallas, no live stock near (same situation as trap No. 4 in previous test); No. 2, 9.7 miles, at laboratory in middle of residential section of Dallas, live stock kept in yard. Southeast, so designated, one trap 7 miles from point of liberation being 2.5 miles east of south, ½ mile northwest of town of Reinhardt, under shed by barn, live stock in yard. Southwest, so designated, one trap 10 miles from point of liberation, 4 miles south of west from that point, placed by empty barn with house, hogpen, and horse lot near by.

The traps to the west, north, east, and south were located on the same roads as in the previous test. The southeast trap was near a main highway running northeast from Dallas to Garland, where it forms a juncture with a macadamized road running east from the point of liberation. Very little if any traffic would pass directly by the point of liberation and around by this trap.

The southwest trap was located on the main highway running northwest from Dallas. The traffic on this road was of about the same character and amount as on the road from Dallas north by the point of liberation. There was practically no chance of any vehicles going from the point of liberation by this trap and no roads giving direct communication between the two points.

On October 10 the first liberation was made. On the preceding day the traps were all placed and baited with fresh slime, as in the preceding experiments. About 25,000 flies colored with unfading red (paint pigment) were released at 11 a. m. These were secured by means of traps set at a packing house on the previous evening. At the time of liberation the sun was partly clouded and a slight breeze was blowing from the south. Many of the flies went several feet into the air and flew in all directions, while others settled on the hedge and vegetation near by. At 4.30 p. m. the same afternoon about 20,000 flies taken in traps at the packing house since the morning were colored in the same way and released. At that hour it was cloudy and cool. The flies did not show a tendency to remain on the cages and on the persons of the writers as in the previous experiment. Most of them settled on the hedge and other vegetation near.

On October 11, at 11 a. m., a third liberation of about 15,000 flies was made. These flies were secured the previous afternoon at the packing house. At 4.30 p. m. an additional lot of about 20,000 flies colored in the same way was liberated. The clouds were heavy and a light shower fell just prior to liberation.

A sample of the flies liberated on the first day was examined to determine the proportion of the species and sex. The results were as follows: *Musca domestica* 66.4 per cent, *Chrysomya macellaria* 28.5 per cent, *Phormia regina* 3 per cent, *Lucilia sericata* 1 per cent, *Ophyra* spp. 0.7 per cent, *Hydrotaea dentipes* Fab. 0.4 per cent. The estimated percentage of species in the lots liberated on the succeeding day agreed closely with these figures.

The approximate averages of these species in the total catch in the 16 recovery traps were: *Musca domestica* 68 per cent, *Chrysomya macellaria* 13 per cent, *Phormia regina* 10 per cent, *Ophyra* spp. 1 per cent, and other species 8 per cent.

Of the 39 marked flies recovered 33.3 per cent were *Musca domestica*, 23.1 per cent *Chrysomya macellaria*, 41 per cent *Phormia regina*, 2.6 per cent *Ophyra leucostoma*. The large percentage of *P. regina* recovered, as compared with the other species, is striking. This may no doubt be explained in part at least by the fact that the cooler temperatures of fall were more favorable for the diffusion and recovery of that species than of the house fly and screw-worm fly. In this experiment, as in the last, the percentage of marked house flies recovered is about one-half of the

percentage of this species liberated and also about one-half of the percentage of this species in the total collection in the recovery traps.

It should be stated that the weather conditions encountered in this test were somewhat adverse, especially as regards variable winds (Table XI). Furthermore, the lateness of the season probably had a markedly adverse influence on the spread of some of the species, notably *Chrysomya macellaria*, which is preeminently a warm-weather species. The test was carried out at a time when the activity and number of *C. macellaria* were declining and the reverse was true in the case of *Phormia regina*, which predominates as a fall and winter species. This is clearly indicated by the diminution in the percentage of these two species of flies taken in the various traps. During the early part of the period the total captures in certain traps averaged about 20 to 40 per cent *C. macellaria* and 7 to 10 per cent *P. regina*, while toward the end of the period the percentage of *C. macellaria* dropped to 5 or 10 and *P. regina* increased to 20 or 30. The total numbers of house flies captured were fairly large throughout the period.

Owing to the distance which it was necessary to traverse in emptying the recovery traps and rebaiting them it was deemed best to make the rounds about every three days, hence for the most part the traps were emptied, rebaited, and the flies examined on October 12, 15, 18, 21, and 29, and others were examined on November 4 and 14. The only notable exception to this arrangement was trap No. 2 south, which was emptied daily after October 15. The bait in this trap was thus given somewhat better attention, and this gave it a slight advantage over the other traps.

DISTANCE OF DISPERSION

The distance the different species of marked flies had traveled is shown in Table X. It will be noted that in general in the nearer traps ranging from 7 to 11 miles the major part of the marked flies were captured. In the more distant traps ranging from 13 to 15 miles only two marked flies were recovered, while in the traps approximately 17 to 18 miles from the point of liberation no liberated flies were taken. The maximum distances recorded for the four species of flies recovered were: *Musca domestica* 13.14 miles, *Chrysomya macellaria* 15.1 miles, *Phormia regina* 10.9 miles, *Ophyra leucostoma* 7 miles.

TABLE X.—*Fourth dispersion test at Dallas, Tex. Species and sex of marked flies recovered in rings of traps about point of liberation*

Trap No.	Distance from place of release.	*Musca domestica.*		*Chrysomya macellaria.*		*Phormia regina.*		*Ophyra leucostoma.*		Total.		Grand total.
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
S. E.	7.0	0	6	0	0	0	2	1	0	1	8	9
1 S.	8.2	0	1	0	1	0	1	0	0	0	3	3
1 W.	9.5	0	0	0	2	0	2	0	0	0	4	4
1 E.	9.3	0	1	0	0	0	0	0	0	0	1	1
2 S.	9.7	0	3	0	1	1	3	0	0	1	7	8
S W.	10.0	0	0	0	2	0	3	0	0	0	5	5
Total		0	4	0	5	1	8	0	0	1	17	18
2 W.	10.9	0	1	0	2	0	3	0	0	0	6	6
2 E.	10.8	0	0	0	0	0	1	0	0	0	1	1
Total		0	1	0	2	0	4	0	0	0	7	7
1 N.	13.14	0	1	0	0	0	0	0	0	0	1	1
3 W.	13.2	0	0	0	0	0	0	0	0	0	0	0
3 E.	13.1	0	0	0	0	0	0	0	0	0	0	0
Total		0	1	0	0	0	0	0	0	0	1	1
4 W.	15.8	0	0	0	0	0	0	0	0	0	0	0
4 E.	15.1	0	0	0	1	0	0	0	0	0	1	1
Total		0	0	0	1	0	0	0	0	0	1	1
5 W.	17.8	0	0	0	0	0	0	0	0	0	0	0
2 N.	16.85	0	0	0	0	0	0	0	0	0	0	0
5 E.	17.0	0	0	0	0	0	0	0	0	0	0	0
Total		0	0	0	0	0	0	0	0	0	0	0
Grand total		0	13	0	9	1	15	1	0	2	37	39

TABLE XI.—*Climatological data relating to fourth dispersion test at Dallas, Tex.*

Date.	Temperature.			Wind.		Rain.	Humidity.			Actual sun.
	Max.	Min.	Mean.	Direction during successive hours from 6 a. m. to 8 p. m.	Velocity during day.		Max.	Min.	Mean.	
1918.	°F.	°F.	°F.		*Miles.*	*Inch.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Oct. 10	76	64	70.0	4 SE., 3 S., 7 NE.	3 to 5	0	90	66	78	8
11	77	64	70.5	3 SE., 5 S., 3 W., 3 N.	2 to 7	0.02	99	72	85	7
12	84	62	73.0	6 E., 2 S., 2 NE., 4 N.	1 to 8	0	100	57	78	70
13	83	64	73.5	2 N., 3 NW., 5 NE., 4 W.	1 to 10	Trace.	87	60	73	67
14	79	59	69.0	3 NE., 3 E., 8 NE.	5 to 16	0	50	25	37	100
15	76	49	62.5	3 NW., 11 NE.	2 to 10	0	74	35	54	94
16	76	62	69.0	2 N., 5 NE., 3 N., 4 NE.	6 to 10	0	66	50	58	35
17	72	63	67.5	4 E., 3 NE., 5 N., 2 NE.	3 to 7	.18	97	84	90	4
18	79	66	72.5	5 N., 2 NE., 2 W., 5 NW.	3 to 7	Trace.	94	66	80	45
19	79	64	71.5	2 NW., 7 W., 2 NW., 3 N.	2 to 7	0	94	78	86	35
20	86	68	77.0	3 NW., 4 N., 2 NW., 5 N.	3 to 9	Trace.	83	50	66	62
21	81	64	72.5	4 E., 4 SE., 3 E., 3 NW.	5 to 10	0	87	64	75	45
22	69	63	66.0	5 SE., 4 S., 2 SE., 3 E.	2 to 17	2.76	100	94	97	0
23	69	62	65.5	1 N., 2 NW., 4 N., 7 NW.	3 to 12	Trace.	97	80	88	18
24	69	62	65.5	11 NW., 3 W.	3 to 11	0	87	66	76	14
25	74	56	65.0	2 E., 11 SE., 1 E.	2 to 13	0	97	60	78	79
26	66	43	54.5	4 SE., 3 N., 7 W.	8 to 20	2.66	99	91	95	0
27	59	42	50.5	12 W., 2 SW.	3 to 18	0	79	56	67	65
28	71	46	58.5	1 SE., 11 S., 2 SE.	8 to 32	0	59	28	43	100
29	67	54	60.5	1 W., 13 NW.	7 to 13	0	89	47	68	55
30	68	47	57.5	10 NW., 4 W.	3 to 10	0	95	47	71	100
31	64	48	56.0	4 W., 6 NW., 4 N.	4 to 10	0	80	37	58	100
Nov. 1	69	42	55.5	10 SE., 1 S., 3 SE.	5 to 13	0	76	29	52	100
2	73	48	60.5	14 SE.	7 to 18	0	72	34	53	94
3	75	52	63.5	4 SE., 6 S., 4 SE.	6 to 12	0	94	60	77	68
4	75	54	64.5	3 SE., 6 S., 5 SE.	5 to 12	0	99	60	79	62
5	76	57	66.5	14 SE.	7 to 18	Trace.	94	75	84	22

DIRECTION OF DISPERSION

The variation in the distances at which the traps in different directions from the point of liberation were located makes it somewhat more difficult to determine the tendencies of the marked flies to travel in different directions. Although the southeast trap was nearest to the point of liberation, it is notable that in it six flies, nearly 50 per cent of the marked house flies, were recovered, the next largest number (4) being in the two traps to the south, one in the east, one in the north, one in the west, and none in the southwest, thus showing with this species a tendency to travel toward the south and southeast. On the other hand, the tendencies as regards direction of flight of *Chrysomya macellaria* and *Phormia regina* appear to differ considerably from those of *Musca domestica*, but are themselves similar. Four (44.4 per cent) *C. macellaria* and five (31.25 per cent) *P. regina* were taken in the west traps, two (22.2 per cent) *C. macellaria* and three (18.75 per cent) *P. regina* in the southwest traps, two (22.2 per cent) *C. macellaria* and five (31.25 per cent) *P. regina* in the south traps, one each (11.1 and 6.25 per cent) in the east traps, and two (12.5 per cent) *P. regina* in the southeast, thus showing in these species a tendency toward migration to the west, southwest, and to a lesser degree to the south.

As is shown in Table XI, the wind during this entire period of recovery was very choppy. During the first 2 days after release there was some south and southeast wind, but aside from this there was during the recovery period a very marked predominance of north and northeast wind. This would seem in the case of the two blowflies to indicate a tendency to travel with the wind in this test. Up to the end of October 16, when recoveries of marked blowflies had nearly ceased, there was experienced during the daylight hours 68 hours of wind from the north, northeast, and east, compared with 17 hours from the west, southwest, and south. The drawing of conclusions on this point, however, would seem unwarranted owing to the extreme variability of the wind during the test.

A considerable number of house flies taken in the southeast trap and of blowflies taken in this and the southwest trap indicates still further that dissemination by following vehicles along the roads is probably not an important factor in this or the previous tests.

In this test, as in the previous one, the variable wind made it difficult to determine whether attractive odors such as those produced by towns and cities are an important influence in determining direction or distance of flight.

RELATION OF SEX TO DISPERSION

In this test all of the 13 marked house flies recovered were females, as were the 9 screw-worm flies and all but 1 (6.25 per cent) of the 16 specimens of *Phormia regina*. The only specimen of *Ophyra leucostoma* taken was a male.

The proportion of sexes among the released flies as indicated by examination of a sample at the time of release was: *Musca domestica*, 34 per cent males, 66 per cent females; *Chrysomya macellaria*, 6 per cent males, 94 per cent females; *Phormia regina*, 22 per cent males, 78 per cent females; *Ophyra leucostoma*, 100 per cent males; *Lucilia sericata*, 100 per cent females. In the case of the house fly we would have expected to recover a few males if that sex had shown migratory tendencies equal to the female, but with *C. macellaria* the percentage of males released was so small and the number of marked flies recovered so few that we would hardly expect to recapture any males.

RELATION BETWEEN POSITION OF TRAP AND NUMBER AND SPECIES OF FLIES CAPTURED

As in the previous test, the number and percentage of different species of flies captured in the different traps varied considerably. During the period covered by the test a total of 12,811 gm. of flies were taken. In this test the average catch per trap of all species was: West, 762 gm.; north, 1,212 gm.; east, 587 gm.; south, 1,229 gm.; southeast, 454 gm.; southwest, 732 gm. The size of the total catches in different traps in either direction varied greatly, especially in the five traps to the west. It appears quite certain that traps set in situations favorable for flies and where a comparatively large catch is obtained would be more likely to capture marked individuals coming to that locality. Furthermore, those traps which are situated most favorably for catching house flies would, other conditions being equal, show a larger percentage of marked house flies as compared with marked blowflies, and the reverse is equally true. This factor probably had some influence on the results in the present test, although the comparatively small number of marked flies captured makes the drawing of conclusions on this point unwarranted.

LONGEVITY OF MARKED FLIES

In addition to the fact that we are concerned with the possible adverse effects of handling and marking the flies used in these various tests, there is presented an opportunity of determining the facts as to the longevity of several species of flies under natural or unrestrained conditions.

It is apparent also that longevity must play an important part in the securing of records on dissemination by the method used in these tests. Since few flies were colored in proportion to the area covered by the recovery traps, especially in the last two tests, and since under such conditions we can hope to capture but a small portion of the flies in the area or even in the vicinity of the traps, each additional day of life and activity of the marked flies enhances the chances of capturing them.

In the several tests the following records of longevity were obtained: First test at Dallas (radius of dispersion 3 miles), *Musca domestica* 11 to

12 days, *Chrysomya macellaria* 7 to 8 days, *Phormia regina* 5 to 6 days, *Lucilia sericata* 5 to 6 days, *Sarcophaga* spp. 11 to 12 days, *Ophyra leucostoma* 3 to 4 days. Second test at Dallas (radius of dispersion 5 miles), *M. domestica* 9 to 11 days, *C. macellaria* 9 to 11 days, *P. regina* 8 to 10 days, *Sarcophaga* spp. 2 to 4 days, *O. aenescens* 6 to 8 days. Third test at Dallas (radius of dispersion 8 miles), *M. domestica* 15 days, *C. macellaria* 17 days, *O. aenescens* 4 days. Fourth test at Dallas (radius of dispersion 17 miles), *M. domestica* 8 to 9 days, *C. macellaria* 10 to 11 days, *P. regina* 10 to 11 days, *O. leucostoma* 1 to 5 days.

In considering the radius of dispersion it should be borne in mind that the marked flies may not necessarily have remained constantly within the circle with the radius given but may have returned into it after more extended travel.

STIMULI AFFECTING DISPERSION

The evidence gained points toward the operation of several stimuli in initiating and governing dispersion. Under the conditions obtaining in nature these stimuli seem to be so blended or mixed as to make it impossible to weigh their relative importance or, in fact, to determine the nature of some of them. It seems probable that the strength of these stimuli and even their character vary with different species.

The importance of food and favorable breeding places as stimuli is clearly shown by the recovery of the greater numbers of specimens of a species in traps located near favorable feeding and breeding grounds. Behind this immediate desire of the individual to obtain food and find a suitable breeding place there appears to be an inherent tendency to disperse. There seems to be some evidence that the house fly was influenced in regard to direction of dispersion by the proximity of farm buildings and towns, indicating spread from one attractive point to another, rather than long sustained flight.

The recovery of males as well as females far from the point of liberation indicates strong dispersion tendencies in that sex as well as in the females. Since mating does not usually take place immediately after emergence, it is no doubt important for the males to seek breeding places where the females are to be found, and they are also attracted to food though not so strongly as are the females.

The question has often been asked, do the flies when liberated tend to return to their original habitat; in other words, is the homing instinct developed? These tests were not planned to determine this point, though they happen to give data on it. The figures presented under the discussion of direction of flight clearly show that there is no marked tendency for any of the species to travel back toward their original place of capture. In this connection it should be remembered that in the first and second tests at Dallas the west traps were nearly in line

from the place of capture to the point of release, and in the third and fourth tests the south traps were about in line.

Climatic influences are unquestionably important in relation to the spread of these species of Diptera. Probably temperature, humidity, sunshine, wind (direction and velocity), precipitation, barometric pressure, and electrical phenomena each exert an influence. It is difficult in experiments of this kind to weigh reliably these various factors because the results are extended over a considerable period with a corresponding change in meteorological conditions, and hence an equalizing of the effects of the stimuli. Those factors which would stimulate activity, such as high temperatures and sunshine, are no doubt potent. Another stimulus is probably to be found in those combinations of climatic conditions experienced with the change of seasons. This seasonal stimulus may account for the marked activity in dispersion and food-seeking found in *P. regina* in the fourth experiment.

COMPARATIVE TENDENCIES OF THE SPECIES TO DISPERSE

The relative tendencies of the several species toward dispersion are suggested by a comparison of the percentages of marked specimens recovered with those liberated and those in the total catch in the recovery traps. These figures indicate that this tendency is more marked in *Chrysomya macellaria* than in *Musca domestica*. The percentage of marked *C. macellaria* as compared with the total marked flies recovered in all of the distant migration tests nearly equaled or surpassed the percentage of that species among the flies liberated, while in *M. domestica* the percentage of marked specimens recovered was approximately one-half of the percentage liberated. Under favorable climatic conditions for the species, *Phormia regina* showed a very strong migratory tendency, the percentage of marked specimens recovered in the third test being 14 times the percentage of that species in the release. *Ophyra leucostoma* and *O. aenescens* also exhibit a decided tendency toward dissemination, the percentage of marked specimens of these species recovered being in all cases greater than in the liberation. The number of specimens of other species liberated was too few to be used in this comparison, but several appear to travel quite freely.

SUMMARY AND CONCLUSIONS

The dispersion of several species of flies important in the economy of man, both as carriers of disease and as parasites of man and animals, is discussed in this paper.

The experiments carried out show that under rural and urban conditions flies have marked powers of diffusion.

The maximum distance of spread from the point of release as recorded in these tests was as follows for the several species: *Musca domestica*,

13.14 miles; *Chrysomya macellaria*, 15.1 miles; *Phormia regina*, 10.9 miles; *Lucilia sericata*, 1.2 miles; *L. caesar*, 3.5 miles; *Synthesiomyia brasiliana*, 0.5 mile; *Sarcophaga* spp., 3 miles; *Ophyra leucostoma*, 7 miles; *O. aenescens*, 4.1 miles.

The estimated total number of marked flies liberated in all the experiments reported upon was 234,000.

In these tests it is considered that too few individuals of species other than *Musca domestica*, *Chrysomya macellaria*, and *Phormia regina* were liberated to form a reliable guide to their dissemination tendencies.

Marked flies of all species dispersed in all directions from the point of liberation.

Among the stimuli inducing dispersion the desire for food and the desire for places for oviposition appear to be among the strongest.

The fact that many towns, farmhouses, and other favorable feeding and breeding grounds were passed by the flies shows that *Musca domestica*, *Chrysomya macellaria*, and *Phormia regina* at least are not satisfied by the mere finding of these places but have marked migratory habits.

Chrysomya macellaria evinces stronger tendencies toward migration than does *Musca domestica*. This tendency in *Phormia regina* under optimum climatic conditions for the species is probably equal to that in *C. macellaria*. The other species were liberated in numbers too few for conclusions to be drawn, but *Ophyra leucostoma* and *O. aenescens* show marked ability to travel considerable distances.

The exact relation between direction of dispersion and direction of wind could not be determined from the results of these experiments because of the choppy wind conditions experienced. There appears to be a tendency for *Musca domestica* and *Chrysomya macellaria* to go with the wind in greatest numbers, but they are shown to travel against and at right angles with it as well. It is concluded that under natural conditions the influence of moderate winds on dissemination is not of great importance.

The evidence gained justifies the conclusion that the passing of vehicles along the highways was not a dominating factor in the dispersion of any species of flies in these tests. This does not mean, however, that flies under other conditions may not be widely scattered by artificial means.

These tests show that the house fly, screw-worm fly, and black blowfly spread rapidly for many miles. *Chrysomya macellaria* was recorded about 8 miles from the point of liberation in less than 24 hours and 10 miles in less than 48 hours after liberation. *Phormia regina* was recovered about 11 miles away in less than 48 hours after release. *Musca domestica* was recovered over 6 miles from the point of release in less than 24 hours.

Males as well as females of the principal species used in these experiments may travel many miles.

The maximum longevity of the marked flies after liberation as shown by the records of capture was: *Musca domestica* 15 days, *Chrysomya macellaria* 17 days, *Phormia regina* 10 to 11 days, *Ophyra aenescens* 6 to 8 days, *Sarcophaga* spp. 11 to 12 days.

While in the fourth experiment no marked flies were captured in the more distant traps (about 17 miles from the point of release), it is the authors' belief that the limits of dispersion were not reached in that test and that where great numbers of flies are emerging constantly the distance traversed may be much farther than the maximum here determined.

The facility with which flies travel many miles emphasizes the importance of the general application of sanitary measures looking toward the suppression of fly breeding.

BACTERIOLOGICAL AND CHEMICAL STUDIES OF DIFFERENT KINDS OF SILAGE

By CHARLES A. HUNTER,[1] *Bacteriologist, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

The aim of this investigation was to study the nature of the fermentations taking place in silage composed of a mixture of silage crops. The investigation was occasioned by the fact that there seems to have been considerable disagreement in the conclusions drawn by different investigators. Through a review of the literature it was noted that plant enzyms were first thought to be the important factors. Recently it has been shown that microorganisms appear to play an important part. Those who favor this latter conclusion claim that the production and increase in acidity are caused by bacterial action. Still more recently some investigators have stated that fermentations were due to the combined action of plant enzyms and microorganisms and that the former of these was the more important factor.

It is definitely known that as silage fermentations take place there is an increase in the volatile and nonvolatile acids and ammonia nitrogen. It has been claimed that the hydrolsis of protein is first caused by enzyms and later by microorganisms.

Corn, kafir corn, cane, corn stover, alfalfa, and alfalfa with a carbohydrate supplement have all been used to make the silage upon which different investigators have worked. In a search of Experiment Station literature the author was unable to find that any bacteriological and chemical studies had been made on silage composed of a mixture of silage crops. It was therefore deemed desirable to study two of the most common silage mixtures now used on Pennsylvania farms—Canada field peas with oats and corn with soybeans. The nature of fermentations in silage made from these two mixtures was studied in the seasons of 1918 and 1919.

REVIEW OF LITERATURE

Babcock and Russell (*1*, *2*)[2] found that silage would undergo fermentation even when treated with chloroform, ether, and benzene which inhibited the growth of microorganisms. They found also that the amount of heat and acids increased and concluded that this was the result of intramolecular respiration. E. J. Russell (*16*) came to nearly the same conclusion as Babcock and Russell in deciding that plant cells and

[1] Special credit is due Prof. S. I. Bechdel, of the Department of Dairy Husbandry, who stored the crops in the large silos, assisted very materially in the securing of the representative samples as they were needed from time to time, helped make the determinations of the total and volatile acids, and gave invaluable assistance in preparing the manuscript.

[2] Reference is made by number (italic) to "Literature cited," p. 788–789.

enzyms were the primary and essential factors in silage fermentation and that bacteria played a minor part.

Esten and Mason (*6*) attributed the fermentation largely to the work of bacteria and yeasts. They concluded that there were three chief fermentations, lactic acid, alcohol, and acetic acid. The primary change was the fermentation of sugar by organisms similar to the common milk-souring organisms which converted it to lactic acid. The secondary change was produced by yeast which converted the remaining sugar to alcohol, the acetic acid bacteria then oxidizing the alcohol to acetic acid.

Samarani (*17*) believed that the sugars were transformed to alcohol and later oxidized to acetic acid by respiration of plant cells. The formation of lactic acid was the result of the action of a bacillus and a coccus which he found in almost equal proportions. He thought the organisms were the same as those that are common in milk fermentation.

Hunter and Bushnell (*13*) conclude that microorganisms cause the major fermentation of silage. They found large numbers of organisms belonging to the bulgaricus group present and state that these organisms are responsible for the high acid content of silage. Organisms belonging to the bulgaricus group and the colon group produce the greater part of acetic acid. Sherman (*18*) has also shown that silage contains large numbers of the organisms belonging to the bulgaricus group.

Lamb (*14*) states that—

> neither microorganisms nor plant enzymes are alone responsible for the changes in silage fermentation.

Microorganisms are largely responsible for the production of acid and the disappearance of sugars. He also states that the formation of alcohols and the hydrolysis of protein as indicated by the amino-nitrogen content are primarily due to cell respiration, although later in the fermentation microorganisms show some activity in both processes.

Hunter (*11*) has shown that the heat production in forage silage is due to microbial activity and not to intramolecular respiration of plant tissue. In his studies on alfalfa silage (*12*) he found that alfalfa alone made a silage of inferior quality but that upon the addition of an available carbohydrate supplement a good quality of silage could be produced. The protein hydrolysis of alfalfa silage as indicated by the amino-nitrogen and ammonia determinations was greater than when a carbohydrate was present. Round (*15*), working on the fermentation of sauerkraut, found that cell respiration plays a more important part than was formerly thought.

Sherman and Bechdel (*19*), working on the fermentation of corn stover silage, found that it undergoes fermentation similar to corn silage. The following statement is also made:

> From a review of the present status of the question as to whether bacteria or plant cells are mainly responsible for silage fermentation, it is concluded that the data thus far published are inconclusive. Although the results of the present study tend to support the cell respiration theory, conclusions on this point are withheld.

METHOD OF PROCEDURE

Two main sets of experiments were planned—first, field experiments; second, laboratory experiments. Several investigations were planned under each set. It was planned to make an analysis at the time of storage, every other day for a week, every three days for the next week, and then once every week until the silage was about a month old. There were times in making these analyses when it was necessary to deviate slightly from the original plans.

SILOS

The two silos used in storing the different kinds of field silage were of the monolithic concrete type with inside dimensions of 8 feet in diameter and 30 feet in height. The walls, 6 inches in thickness, were nonporous and the inside was made smooth with a coat of plaster. To afford a means of sampling, pieces of 2-inch water pipe 6 inches long were placed in the wall at the time the silos were constructed. These sample holes were located 3 to 5 feet apart all the way around the silos to a height of 12 feet and were plugged.

The samples used in all tests were obtained by making repeated borings through these holes with a 2-inch auger. A new hole was used for obtaining material for each successive analysis. The auger was provided with an extension shaft 8 feet in length so as to penetrate to the center of the silo.

The silos used for laboratory experiments consisted of quart milk bottles, stoppered with rubber stoppers wired in, and sealed with paraffin. One bottle of silage was used for each day's analysis.

BACTERIOLOGICAL METHODS

Samples of silage were collected from the concrete silos in sterile containers and taken to the laboratory. In order to secure a representative sample the silage was passed through a sterile meat grinder. Twenty gm. of this silage were placed in 200 cc. of sterile physiological salt solution. This was thoroughly shaken, and further dilutions were made.

TOTAL NUMBER OF MICROORGANISMS.—In determining the total number of bacteria, plain agar was used. No doubt a somewhat greater number of organisms could have been obtained with a carbohydrate medium, but the relative increase or decrease in the number would have been the same.

TOTAL ACID PRODUCERS.—Dextrose litmus broth and dextrose cresol purple broth were used. Dextrose litmus broth was prepared by using 1 per cent dextrose broth to which litmus had been added. Cresol purple solution was made by dissolving 0.4 gm. of di-bromo-ortho-cresol-sulphonphthalein in a minimum amount of alcohol and making up to 1 liter with water. Forty cc. of this were added to 1 liter of dextrose broth. The broths were inoculated with different dilutions of the silage

infusion and incubated. Acid formation in the cresol purple broth is indicated by a yellow color. This color was very distinct, and only a trace of acid present caused the broth to change from purple to yellow. In litmus the results were at times rather indistinct; moreover, the litmus was not as sensitive to small amounts of acid as cresol purple. Cresol purple was used 50 times in comparison with litmus. Cresol purple gave more organisms 14 times; litmus gave higher results 9 times; and equal results were obtained 27 times. Expressed in percentage, the greater number of bacteria were found in the cresol purple solution in 28 per cent of the times, in litmus 18 per cent, and in equal numbers 54 per cent.

COLON-AEROGENES GROUP.—Bile lactose broth and lactose broth were used to determine the number of colon organisms present. Dunham fermentation tubes containing approximately 10 cc. of respective media were inoculated with 1 cc. of various dilutions of the silage infusions. The number of organisms present was determined from the tube showing gas in the highest dilution. In order to verify the presumptive test, colon organisms were isolated at different intervals. Bile lactose broth and lactose broth were used 55 times in comparison with each other. Lactose broth gave more organisms 8 times, bile lactose broth gave more 11 times, and equal results were obtained 36 times. In terms of percentage, lactose broth gave the greater number of organisms in 14.6 per cent of the determinations, bile lactose in 20 per cent, and equal results were obtained in 65.4 per cent.

BULGARICUS.—The plate method was used to determine the number of bulgaricus organisms present. One per cent dextrose agar was used to which 1 cc. of a 1 per cent solution of sterile acetic acid was added at the time the plates were poured. From time to time organisms belonging to the bulgaricus group were isolated and identified.

YEAST.—The dilution method was used to determine the number of yeasts present. One per cent dextrose broth in Dunham fermentation tubes was inoculated with 1 cc. of the dilutions of silage extract. The formation of gas was taken as an indication that yeasts were present. This, however, was confirmed by microscopic examination.

PROTEIN DIGESTERS.—Gelatin was inoculated with 1 cc. of the various dilutions and incubated at 37° C. The gelatin upon being removed from the incubator was placed in a refrigerator. Gelatin which has been digested will not solidify upon cooling; hence by taking the highest dilution showing liquefaction the number of protein digesters could be determined.

All media used were made according to standard methods and were titrated with brom-thymol blue (di-brom-thymol-sulphonphthalein). This gave the media a hydrogen concentration of 6.4–7.6 (*4*). The period of incubation was seven days at 37° C. Direct microscopic counts of the silage juice were made with Breed's method. The results obtained compared very favorably with those secured by the plate method.

CHEMICAL METHODS

From the sample of silage the juice was expressed and used for the chemical analysis.

TOTAL ACIDITY.—Ten cc. of juice were diluted with 990 cc. of distilled water and titrated with *N/10* barium-hydroxid solution, using phenolphthalein as an indicator.

VOLATILE ACIDITY.—Twenty-five cc. of juice were subjected to steam distillation under reduced pressure. One liter of distillate was secured and titrated immediately with *N/10* barium hydroxid, using phenolphthalein as an indicator. Experiences in other investigations have established the efficiency of this method (*5*).

AMINO NITROGEN.—The amino nitrogen was determined by the use of the Van Slyke apparatus (*20*).

AMMONIA NITROGEN.—The ammonia was determined by the use of Folin's method, using 25 cc. of juice and aerating for at least eight hours.

ALBUMINOID NITROGEN.[1]—This was run on the first and last sample of most of the silage. It was determined by Stutzer's method.

TOTAL NITROGEN.[1]—The total nitrogen was determined by the Kjeldahl method.

MOISTURE.—One thousand gm. of a well-mixed sample of the silage were placed in an air-drying oven for at least three days.

FIELD EXPERIMENTS

Three separate field experiments were performed:

I. Canada field peas and oats, 1918.
II. Canada field peas and oats, 1919.
III. Corn in comparison with corn and soybean mixture, 1918.

EXPERIMENT I. CANADA FIELD PEA AND OAT SILAGE, 1918

The crop of oats and peas was seeded May 6 and stored in the silo July 17. The oats were in the milk stage of growth and the peas were well formed in the pod. The fresh green mixture at the time of ensiling contained, on the average, 18.4 per cent peas. On account of the extremely dry weather for the two weeks just previous to harvesting, the moisture content was rather low, being only 70 per cent. The silage mixture kept very well and was eaten with relish by the cows on the experiment. Feeding was not begun until August 8 so as not to interfere with the method of securing samples. The results of the bacteriological examination are given in Table I.

[1] The analytical work of determining the total and albuminoid nitrogen was done by Mr. Walter Thomas, Assistant Chemist of the Pennsylvania Agricultural Experiment Station.

TABLE I.—*Results of bacteriological examination of Canada field pea and oat silage in concrete silo*

[Expressed in number of bacteria per gram]

Date sampled.	Number of days from ensiling.	Total in plain agar.	Total acid producers.		Colon group.		Bulgaricus group.	Yeast.	Liquefiers in gelatin.
			Dextrose litmus broth.	Cresol purple broth.	Bile lactose broth.	Lactose broth.			
1918.									
July 19	0	1,800,000	100,000,000	1,000,000,000	1,000,000	10,000,000	600	10,000,000	1,000
20	1	550,000,000	100,000,000	100,000,000	100,000,000	100,000,000	265,000	100,000	10,000,000
23	4	150,000,000	100,000,000	10,000,000	710,000,000	710,000,000	25,000,000	100,000	100,000
25	6	97,000,000	1,000,000	1,000,000	10,000	10,000	735,000	100,000	100,000
27	8	280,000,000	100,000,000	100,000,000	710,000,000	10,000,000	.....	10,000	100,000,000
30	11	.....	1,000,000	10,000,000	0	0	18,500	1,000,000	1,000,000
Aug. 3	15	.....	10,000,000	1,000,000	100,000	100,000	.....	100,000	1,000,000
7	19	3,500,000	10,000,000	10,000,000	10,000,000	10,000,000	543,000	10,000	10,000
14	26	6,900,000	10,000,000	10,000,000	10,000,000	10,000	31,000	100,000	10,000

From the results in Table I it can be noted that the oats and Canada field peas at the time of ensiling contained a large number of organisms. The acid-producing organisms were predominating. Organisms belonging to the bulgaricus group were present in very small numbers. Yeasts and organisms belonging to the colon group were present in large numbers. There were few protein digesters present at the time of ensiling. As fermentation progressed the number of acid formers remained fairly constant for the first eight days and then slightly decreased. The bulgaricus group increased rapidly for the first four days; then there was a gradual decrease. The number of yeast and protein digesters did not decrease as they did in some of the later experiments. One reason, no doubt, was the fact that the silage material was cut very coarse so that the silage did not compact properly, and air pockets were formed. The count upon plain agar shows an increase, then a decrease. The number of organisms belonging to the colon group increased, disappeared on the eleventh day, and then increased.

It is true that the changes which apparently took place in the silage might possibly be ascribed to variations in the samples of silage which were taken from different parts of the silo, but on account of the progressive nature of the changes taking place as shown by the increase in numbers of bacteria and in the formation of chemical compounds it would seem probable that the changes were due to the agents causing the fermentation.

EXPERIMENT II. CANADA FIELD PEA AND OAT SILAGE, 1919

The crop in 1919 was seeded earlier than in 1918 and was ready for the silo on July 3. The growing season was also very dry in 1919 until about two weeks before harvesting, and as a result the crop yield was low. The moisture content of the fresh green material was 73.8 per cent, or a little higher than that of the 1918 crop. The percentage of peas was also slightly higher, being 21.1 per cent in this investigation. A feeding test was conducted in which the silage was compared with ordinary corn silage for milk production. It proved to be very good feed, and the cows consumed it in large quantities with very good results in milk production.

Table II shows the bacteriological results obtained from this experiment. It can be noticed that the predominating group of organisms were the acid producers, of which a large percentage, no doubt, belonged to the bulgaricus group. After the first day of fermentation practically no organisms belonging to the colon group nor any yeasts were present. There were few protein digesters present.

The chemical results given in Table III show a decided increase in the total acidity, volatile acidity, and amino nitrogen during the first few days of fermentation. This was then followed by a gradual increase until the twenty-ninth day, when analyses were stopped. Ammonia determination fluctuated but showed gradual increase.

TABLE II.—*Results of bacteriological examination of Canada field pea and oat silage in concrete silo*

[Expressed in number of bacteria per gram]

Date sampled.	Number of days from ensiling.	Total in plain agar.	Total acid producers in cresol purple broth.	Colon group.		Bulgaricus group.	Yeasts.	Liquefiers in gelatin.
				Bile lactose broth.	Lactose broth.			
1919.								
July 3	0	Less than 10,000	1,000,000	1,000	1,000	Less than 100	100	1,000
7	4	1,580,000	100,000,000	0	0	250,000	10	10,000
9	6	73,000	100,000,000	0	0	1,760,000	0	10,000
11	8	.....	10,000,000	0	10	1,740,000	100	10,000
14	11	3,000,000	100,000,000	0	0	225,000	0	10,000
16	13	40,000	100,000	0	100	290,000	10	.....
18	15	350,000	100,000	0	0	275,000	0	100
25	22	.....	100,000	0	0	250,000	0	10
Aug. 1	29	2,000,000	10,000,000	0	10	4,000,000	0	7,000

TABLE III.—*Results of chemical analyses of Canada field pea and oat silage in concrete silo*

[Expressed in milligrams per 100 cc. of juice]

Date sampled.	Number of days from ensiling.	Moisture.	Total acidity in terms of lactic acid.	Volatile acidity in terms of acetic acid.	Nonvolatile acidity by difference.	Amino nitrogen.	Ammonia nitrogen.	Milligrams albuminoid nitrogen in 100 gm. dry silage.	Milligrams nitrogen in 100 gm. dry silage.
1919.		*Per cent.*							
July 3	0	73.82	984.5	29.23	955.27	176.5	54.4	95.0	157.0
7	4	62.7	3,199.30	663.8	2,535.50	271.4	54.4	.....	166.5
9	6	70.8	3,222.18	625.0	2,597.18	322.7	65.2	.....	168.0
11	8	72.9	3,288.81	604.1	2,684.71	257.6	44.8	.....	154.0
14	11	67.3	3,641.85	806.9	2,834.95	389.7	88.4	.....	183.0
16	13	70.6	3,376.32	769.66	2,579.66	314.7	61.2	.....	164.0
18	15	71.2	3,339.53	751.76	2,587.77	333.2	69.3	.....	157.0
25	22	70.9	3,377.32	818.88	2,558.44	339.1	65.2	.....	156.0
Aug. 1	29	66.7	3,759.21	1,005.3	2,753.91	416.5	76.84	79.5	180.5

It is of interest to note that the decided increase in number of acid producers and the decided increase in acidity occur at the same time, thus indicating that acidity is due to micro-organisms.

EXPERIMENT III. CORN SILAGE AND CORN AND SOYBEAN SILAGE, 1918

The same variety of corn was grown in each case. The corn-soybean mixture was obtained by planting soybeans with a hand seeder in the same rows with the corn. In proportion by green weight the soybeans in the corn-soybean mixture amounted to approximately 30 per cent. As large a percentage of soybeans as this could not be hoped for under ordinary conditions, since the viability of seed corn used was rather low, and the number of stalks was little more than half that of a normal stand.

Ensiling took place when the corn was well glazed and when the soybeans were well podded. It was necessary to add some water to the corn silage to insure its preservation, as it was somewhat advanced in maturity.

On account of the rainy season both kinds of silage contained an unusual amount of weeds. It is not believed that this affected the quality materially, since in a feeding test which followed the animals relished and ate them both throughout the experiment with practically no waste.

This experiment was run for the purpose of comparing corn silage with silage composed of a mixture of corn and soybeans. (Tables IV to VII.)

The bacteriological results given in Tables IV and VI show little difference between the two kinds of silage.

Acid-producing organisms were the predominating group. For the first few days there were large numbers of organisms belonging to the colon group, but there was a gradual reduction until on the thirty-ninth day, when there were no colon organisms in the corn silage and only a few in the corn and soybean silage. There was a gradual reduction in the number of yeasts and protein digesters in both kinds of silage.

The chemical analyses given in Tables V and VII show that the greatest increase in acidity and amino nitrogen occurs during the first four days of the fermentation. The ammonia determinations fluctuate to a considerable extent, with a tendency to decrease in the corn silage and increase in the corn and soybean silage.

Graphs showing the increase in acidity, amino nitrogen, and ammonia are given in figures 1, 2, and 3, respectively.

LABORATORY EXPERIMENTS

Three laboratory experiments were performed with Canada field pea and oat silage:

IV. Untreated.

V. Treated with 2 per cent chloroform.

VI. Sterilized and inoculated.

TABLE IV.—*Results of bacteriological examination of corn silage in concrete silo*

[Expressed in number of bacteria per gram]

Date sampled.	Number of days from ensiling.	Total in plain agar.	Total acid producers.		Colon group.		Bulgaricus group.	Yeast.	Liquefiers in gelatin.
			Dextrose litmus broth.	Cresol purple broth.	Bile lactose broth.	Lactose broth.			
1918.									
Sept. 9	0	30,000,000	10,000,000	10,000,000	10,000,000	100,000	4,400	10,000	1,000,000
10	1	23,000,000	100,000,000	100,000,000	10,000,000	1,000,000	1,670,000	1,000	100,000
12	3	880,000,000	1,000,000,000	100,000,000	1,000,000	1,000,000	417,000,000	100,000	1,000,000
13	4	590,000	1,000,000,000	1,000,000,000	100,000	100,000	162,000,000	10,000	100,000
14	5	130,000	100,000,000	1,000,000,000	100,000	100,000	14,000,000	10,000	10,000
16	7	65,000,000	1,000,000,000	100,000,000	10,000	100,000	7,000,000	.....	10,000
18	9	.....	1,000,000	100,000,000	10,000	10,000	15,200,000	10,000	.....
20	11	3,500,000	.....	100,000,000	10,000	10,000	43,000,000	.....	1,000
27	18	.....	.....	100,000,000	100	100	33,000,000	.....	10,000
Oct. 18	39	360,000	.....	1,000,000,000	0	0	17,000,000	.....	.....

TABLE V.—*Results of chemical analysis of corn silage in concrete silo*

[Expressed in milligrams per 100 cc. of juice]

Date sampled.	Number of days from ensiling.	Moisture.	Total acidity in terms of lactic acid.	Volatile acidity in terms of acetic acid.	Nonvolatile acidity by difference.	Amino nitrogen.	Ammonia nitrogen.	Milligrams albuminoid nitrogen in 100 gm. dry silage.	Milligrams total nitrogen in 100 gm. dry silage.
1918.		*Per cent.*							
Sept. 9	0	69.0	447.6	.....	.....	69.1	18.0	77.0	125
10	1	71.84	671.4	.....	.....	86.3	14.28	.....	146
12	3	68.34	.....	.....	.....	.....	.....	.....	.....
13	4	66.2	2,293.95	208.8	2,085.15	124.5	17.0	.....	131
14	5	65.5	2,014.20	.....	.....	152.3	8.84	.....	157
16	7	65.0	2,629.65	268.5	2,361.15	176.1	13.92	.....	152
18	9	67.2	2,685.6	220.77	2,464.83	172.1	15.64	.....	133
20	11	66.4	2,831.07	313.23	2,517.84	149.1	5.96	.....	152
27	18	67.8	2,853.45	.....	.....	.....	.....	.....	.....
Oct. 18	39	69.7	2,942.97	.....	.....	176.1	.....	69.5	128

TABLE VI.—*Results of bacteriological examination of corn and soybean silage in concrete silo*

[Expressed in number of bacteria per gram]

Date sampled.	Number of days from ensiling.	Total in plain agar.	Total acid producers.		Colon group.		Bulgaricus group.	Yeast.	Liquefiers in gelatin.
			Dextrose litmus broth.	Cresol purple broth.	Bile lactose broth.	Lactose broth.			
1918.									
Sept. 9	0	45,000,000	10,000,000	1,000,000	1,000,000	10,000	28,500	10,000	1,000,000
10	1	220,000,000	100,000,000	100,000,000	10,000,000	1,000,000	58,000	1,000	1,000,000
12	3	500,000,000	1,000,000,000	1,000,000,000	10,000,000	100,000	630,000,000	10,000	10,000
13	4	1,100,000	10,000,000,000	10,000,000,000	100,000	1,000,000	25,000,000	10,000	100,000
14	5	400,000	100,000,000	100,000,000	100,000	1,000,000	60,000,000	10	1,000
16	7	610,000	1,000,000	1,000,000,000	1,000,000	100,000	28,000,000	1,000	100,000
18	9	.....	.....	100,000,000	10,000	10,000	6,400,000	100	.....
20	11	1,000,000	.....	100,000,000	10,000	10,000	16,000,000	.....	100
27	18	.....	.....	1,000,000,000	0	0	180,000,000	.....	1,000
Oct. 18	39	380,000	.....	10,000,000	100	100	29,000,000	.....	.....

TABLE VII.—*Results of chemical analysis of corn and soybean silage in concrete silo*

[Expressed in milligrams per 100 cc. of juice]

Date sampled.	Number of days from ensiling.	Moisture.	Total acidity in terms of lactic acid.	Volatile acidity in terms of acetic acid.	Nonvolatile acidity by difference.	Amino nitrogen.	Ammonia nitrogen.	Milligrams albuminoid nitrogen in 100 gm. dry silage.	Milligrams total nitrogen in 100 gm. dry silage.
1918.		*Per cent.*							
Sept. 9	0	69.0	525.9	.....	.....	52.4	12.92	75.0	133
10	1	71.34	760.9	.....	.....	74.2	8.72	.....	157
12	3	63.1	.....	.....	.....	.....	.....	.....	.....
13	4	62.9	1,846.3	229.7	1,616.6	120.7	10.88	.....	136
14	5	57.4	1,734.4	.....	.....	136.2	11.88	.....	134
16	7	62.2	2,036.5	480.2	1,556.3	195.6	10.52	.....	130.5
18	9	59.2	2,282.7	366.9	1,915.8	171.7	25.16	.....	138
20	11	62.7	2,327.5	322.1	2,005.4	191.4	20.4	.....	128
27	18	63.6	2,965.3	.....	.....	.....	.....	.....	.....
Oct. 18	39	66.0	2,998.9	384.8	2,614.1	216.4	17.0	67.5	119

It was thought that bacteriological and chemical results from silage treated as above might throw some additional light on the much debated

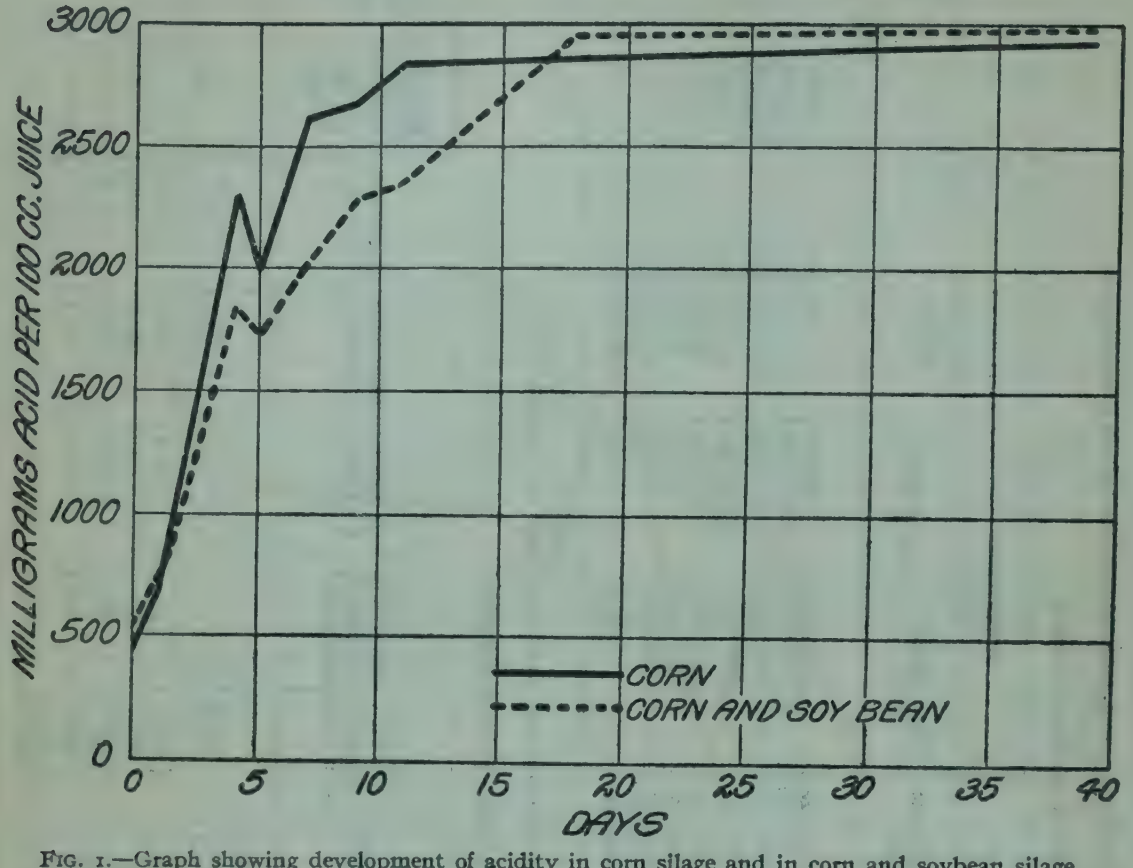


FIG. 1.—Graph showing development of acidity in corn silage and in corn and soybean silage.

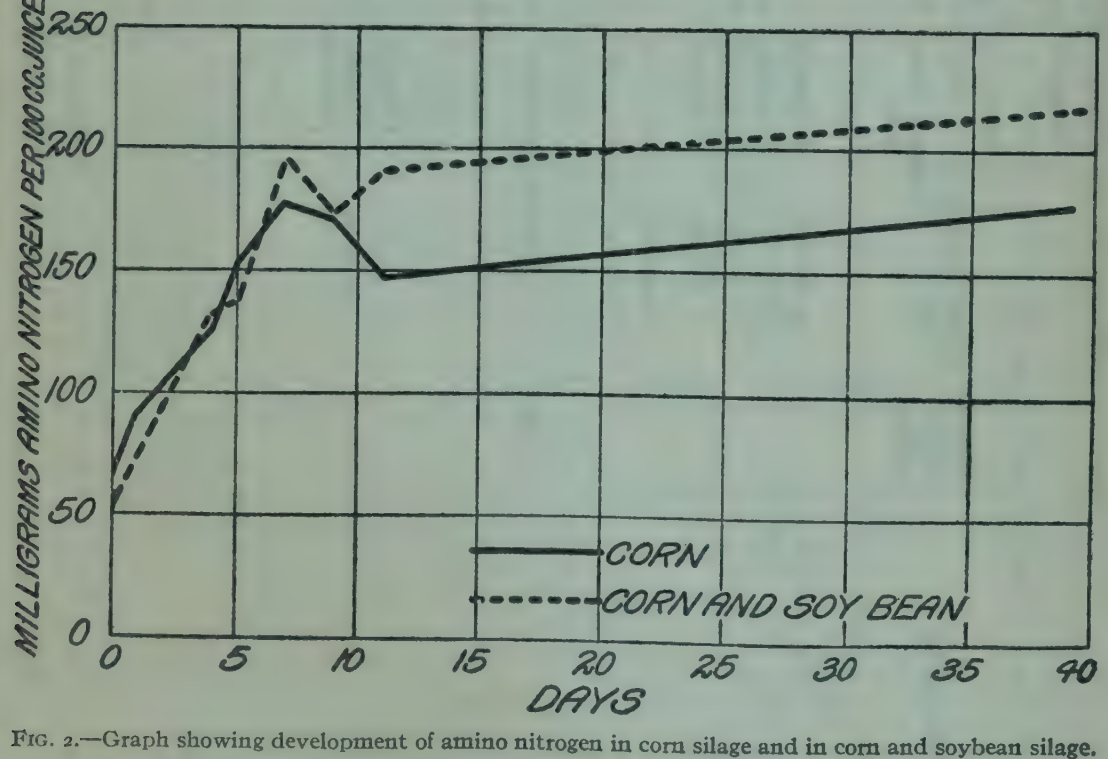


FIG. 2.—Graph showing development of amino nitrogen in corn silage and in corn and soybean silage.

question as to whether microorganisms or plant enzyms are the more important factors in silage fermentation.

EXPERIMENT IV

This experiment was performed at the same time and with the same crop as experiment I. This was done to compare the results of the large silo with a small laboratory silo. The material used in filling the

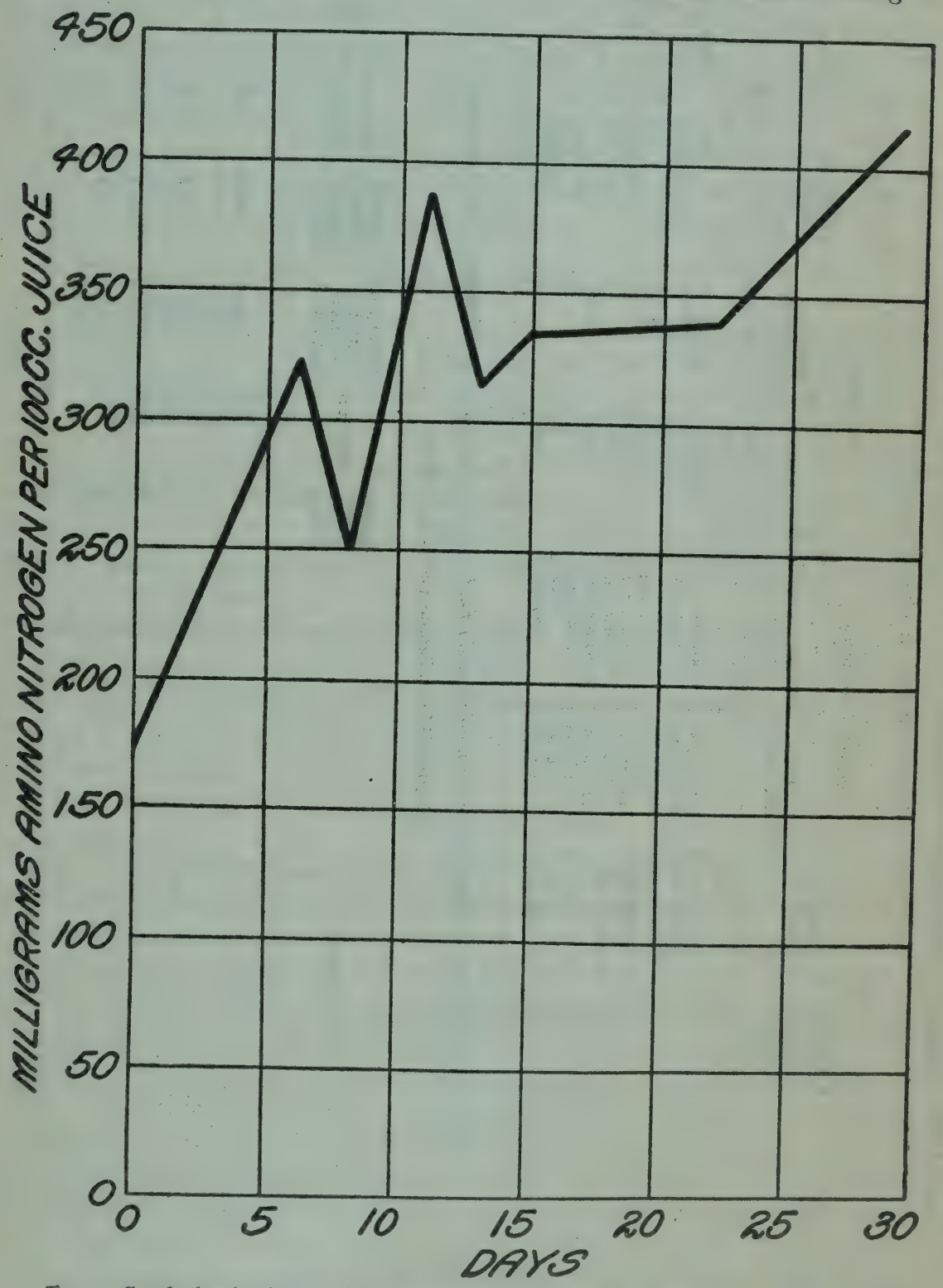


FIG. 3.—Graph showing increase in amino nitrogen in Canada field pea and oat silage, 1919.

laboratory silos was passed through a small grinder as it came from the silage cutter so as to make it sufficiently fine to be used in the small containers.

TABLE VIII.—*Results of bacteriological examination of Canada field pea and oat silage in laboratory silo (untreated)*

[Expressed in number of bacteria per gram]

Date sampled.	Number of days from ensiling.	Total in plain agar.	Total acid producers.		Colon group.		Bulgaricus group.	Yeast.	Liquefiers in gelatin.
			Dextrose litmus broth.	Cresol purple broth.	Bile lactose broth.	Lactose broth.			
1918.									
July 19	0	1,800,000	100,000,000	1,000,000,000	1,000,000	10,000,000	600	10,000,000	1,000
20	1	336,000,000	100,000,000	100,000,000	1,000,000	10,000,000	441,000	1,000	10
23	3	84,000,000	10,000,000	100,000,000	0	0	16,000,000	0	1,000
25	5	550,000	100,000	10,000,000	10,000	10,000	.....	10	10,000
27	7	9,900,000	10,000,000	10,000,000	1,000	10	109,000	10,000	1,000
30	10	5,500,000,000	100,000	1,000,000	1,000	0	1,000,000	10	0
Aug. 1	12	.....	10,000,000,000	1,000,000,000	1,000	1,000	.....	.....	.....
3	14	.....	100,000	1,000,000	0	0	282,000	0	100
7	18	55,000	10,000	10,000	0	0	750	0	100
14	25	180,000	100,000	100,000	100	0	138,000	0	0

TABLE IX.—*Results of chemical analysis of Canada field pea and oat silage in laboratory silo (untreated)*

[Expressed in milligrams per 100 cc. of juice]

Date sampled.	Number of days from ensiling.	Moisture.	Total acidity in terms of lactic acid.	Volatile acidity in terms of acetic acid.	Nonvolatile acidity, by difference.	Amino nitrogen.	Ammonia nitrogen.
1918.		*Per cent.*					
July 19	0	70.0	795.6	100.09	695.51	134.8	5.1
20	1	76.4	1,082.9	.....	.....	183.0	.....
23	3	85.2	2,873.0	.....	.....	261.0	60.84
25	5	76.0	2,541.5	350.3	2,191.2	213.1	55.76
27	7	76.8	2,986.3	.....	.....	238.1	.....
30	10	70.5	2,652.0	.....	.....	247.38	40.06
Aug. 1	12	61.4	2,762.5	500.5	2,262.0	251.2	62.80
3	14	73.2	.....	.....	.....	293.1	44.42
7	18	.....	2,818.0	571.1	2,246.9	307.3	49.98
14	25	87.1	2,828.0	.....	.....	279.6	.....

The bacteriological results given in Table VIII are characteristic of silage fermentation. The acid-producing organisms predominate. There was a variation in number of organisms belonging to the bulgaricus group. There were a large number of yeasts at the time of siloing, but a decided decrease occurred, and in a few days only a few were present. The number of gelatin liquefiers were few at time of storing and gradually decreased until there were no organisms belonging to this group after 25 days. Large numbers of organisms belonging to the colon group were present for the first day but then decreased rapidly.

Table IX gives the chemical results which show an increase in acidity, amino nitrogen, and ammonia as fermentation progresses. The greatest increase occurred during the first three days of fermentation.

EXPERIMENT V

The object of this experiment was to find out the effect of 2 per cent chloroform upon the bacterial flora and the chemical changes brought about by cell respiration. It was also desired to ascertain if silage so treated would undergo normal fermentation. (Tables X and XI.)

Table X gives the bacteriological results of Canada field pea silage treated with chloroform. From the results in this table it can be readily seen that chloroform was very efficient in inhibiting the growth of the microorganisms. After the first day there were few organisms belonging to any of the groups.

The chemical results are given in Table XI and show that there was very little increase in acidity. This indicates that the acidity in silage is due chiefly to microorganisms and is not due to plant enzyms. There was an increase in amino nitrogen and ammonia, showing that the hydrolysis of protein was principally due to cell respiration.

EXPERIMENT VI

Since an experiment had been planned to inhibit the growth of microorganisms, thus allowing cell respiration to play the principal part in fermentation, it was decided to perform one in which plant enzyms were destroyed, allowing action of only the microorganisms. A series of laboratory silos were filled with Canada field peas and oats and sterilized in autoclave for three hours. Each silo was then inoculated with fresh silage juice containing large numbers of microorganisms. (Tables XII and XIII.)

The bacteriological results with Canada field pea silage which was sterilized and inoculated are given in Table XII. They compare very favorably with those with normal silage, there being little difference. The first few days there was an increase in the total number of organisms, followed by a decrease. The acid-producing organisms constituted the main flora, and the number of these organisms decreased with the age of the silage. The number of organisms belonging to the colon group, yeast, and protein digesters decreased rapidly and after a few days disappeared. The number of organisms belonging to the bulgaricus group increased for the first five days and then gradually decreased.

TABLE X.—*Results of bacteriological examination of Canada field pea and oat silage in laboratory silo (treated with 2 per cent chloroform)*

[Expressed in number of bacteria per gram]

Date sampled.	Number of days from ensiling.	Total in plain agar.	Total acid producers.		Colon group.		Bulgaricus group	Yeast.	Liquefiers in gelatin.
			Dextrose litmus broth.	Cresol purple broth.	Bile lactose broth.	Lactose broth.			
1918.									
July 19	0	1,800,000	100,000,000	1,000,000,000	1,000,000	10,000,000	600	10,000,000	1,000
20	1	367,000	100,000,000	10,000,000	7,100,000	100,000	32,000	0	10
22	3	70,000	10,000	10,000	0	10	20	0	100
24	5	600	100	100	0	0	10	0	10
26	7	750	100	100	0	0	0	0	100
29	10	1,800	100	1,000	0	0	40	0	10
31	12	.....	10,000	1,000	100	71,000	.....	1,000	71,000
Aug. 2	14	700	100	100	0	10	.....	.....	100
6	18	900	10,000	100,000	0	0	6,180	0	1,000
13	25	800	100	100	0	0	45	0	100

TABLE XI.—*Results of chemical analysis of Canada field pea and oat silage in laboratory silo (treated with 2 per cent chloroform)*

[Expressed in milligrams per 100 cc. of juice]

Date sampled.	Number of days from ensiling.	Moisture.	Total acidity in terms of lactic acid.	Volatile acidity in terms of acetic acid.	Nonvolatile acidity, by difference.	Amino nitrogen.	Ammonia nitrogen.
1918.		*Per cent.*					
July 19	0	70.0	795.6	100.09	695.51	134.8	5.1
20	1	76	718.2	.....	.....	145.5	.....
22	3	79.4	663.0	.....	.....	197.5	10.54
24	5	73.6	751.4	.....	.....	231.2	51.00
26	7	75.3	663.0	53.04	609.96	216.9	42.5
29	10	70.8	773.5	.....	.....	221.3	42.7
31	12	69.9	497.2	255.6	241.6	203.8	44.2
Aug. 2	14	80.1	442.0	.....	.....	169.9	.....
6	18	74.4	773.5	147.3	626.5	236.0	37.25
13	25	86.5	895.0	.....	.....	269.5	38.76

TABLE XII.—*Results of bacteriological examination of Canada field pea and oat silage in laboratory silo (sterilized and inoculated)*

[Expressed in number of bacteria per gram]

Date sampled.	Number of days from ensiling.	Total in plain agar.	Total acid producers.		Colon group.		Bulgaricus group.	Yeast.	Liquefiers. in gelatin.
			Dextrose litmus broth.	Cresol purple broth.	Bile lactose.	Lactose broth.			
Inoculating juice		40,000,000	100,000,000	100,000,000	1,000,000	1,000,000	100	10,000	10
1918.									
July 20	1	345,000,000	1,000,000	1,000,000,000	1,000,000	1,000,000	46,000	0	0
22	3	310,000,000	1,000,000	10,000,000	0	0	250,000	0	0
24	5	270,000,000	100,000	10,000,000	0	0	18,000,000	0	0
26	7	1,400,000	1,000,000	10,000	0	0	5,440,000	0	0
29	10	1,500,000	10,000,000	10,000,000	0	10	.....	0	10,000
31	12	.....	10,000,000	10,000,000	10	10	.....	0	0
Aug. 2	14	520,000	71,000,000	71,000,000	0	0	.....	0	0
6	18	2,970,000	1,000,000	1,000,000	0	0	130,000	0	0
13	25	125,000	100,000	100,000	0	0	105	0	10

TABLE XIII.—*Results of chemical analysis of Canada field pea and oat silage in laboratory silo (sterilized and inoculated)*

[Expressed in milligrams per 100 cc. of juice]

Date sampled.	Number of days from ensiling.	Moisture.	Total acidity in terms of lactic acid.	Volatile acidity in terms of acetic acid.	Nonvolatile acidity by difference..	Amino nitrogen.	Ammonia nitrogen.
		Per cent.					
1918.							
July 19	0	70.0	795.6	100.9	695.51	38.4	5.1
20	1	77.0	828.7	.....	.....	57.4	.....
22	3	77.2	1,436.6	.....	.....	50.1	10.2
24	5	86.4	1,524.9	238.6	1,286.3	66.6	36.7
26	7	76.2	1,679.6	.....	.....	58.8	31.28
29	10	73.8	2,088.4	536.3	1,552.1	67.5	22.1
31	12	60.5	2,121.6	.....	.....	60.0	22.1
Aug. 2	14	.....	2,232.1	447.9	1,784.2	72.2	17.68
6	18	73.0	1,712.7	.....	.....	59.8	19.5
13	25	83.4	2,419.9	.....	.....	74.7	28.28

Inasmuch as microorganisms are the only agents concerned in the experiment, the results of the chemical analyses given in Table XIII show that the acidity was due chiefly to their activity. Part of the decomposition of protein with formation of ammonia was due to the action of microorganisms. Microorganisms played very little part in the formation of amino nitrogen.

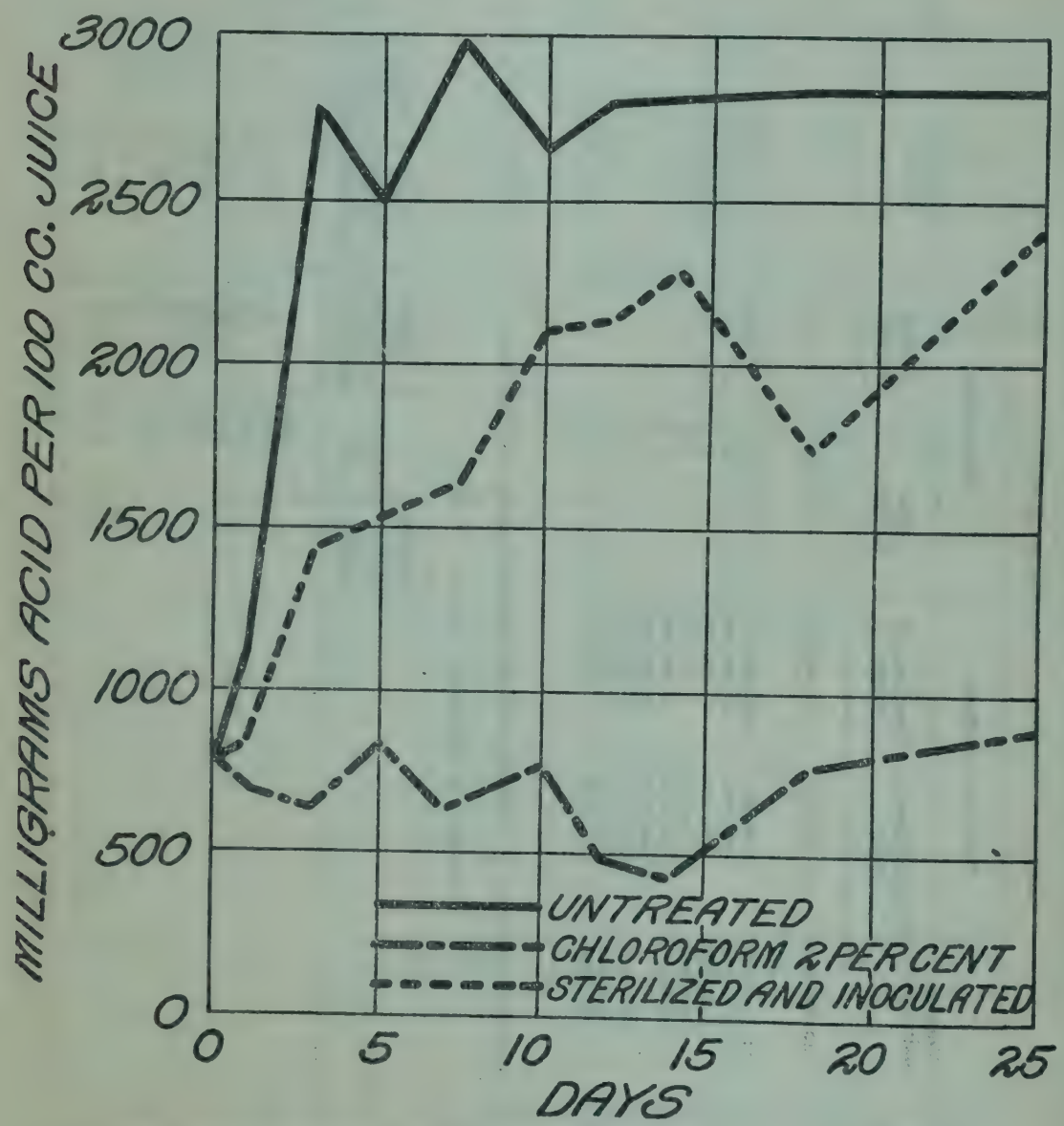


FIG. 4.—Graph showing increase in acidity in Canada field pea and oat silage, 1918.

In order that the increase in acidity, amino nitrogen, and ammonia in the laboratory experiments may be more easily compared, the results are given in figures 4, 5, and 6, respectively.

DISCUSSION OF RESULTS

The results of bacteriological and chemical analyses of various kinds of silage indicate that microorganisms are the principal agents in the fermentation of silage. It is quite natural to assume that enzyms would play some part in the fermentation, and it was found that enzyms did play a part in the hydrolysis of protein.

The production of acidity appears to be due almost entirely to micro-organisms. Silage treated with 2 per cent chloroform, thus inhibiting growth of bacteria, showed practically no increase in acidity, while silage sterilized and inoculated, which process destroyed plant enzyms,

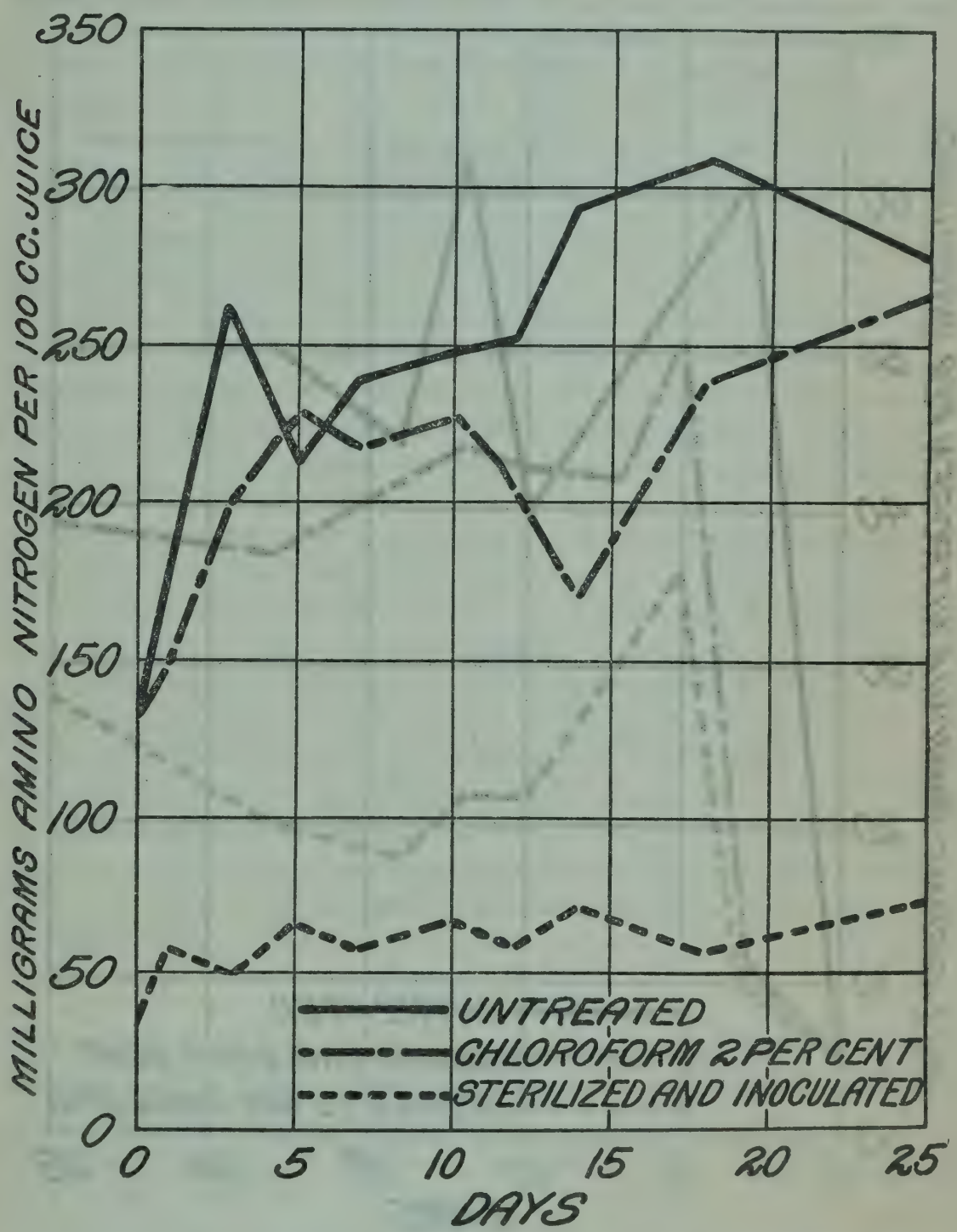


FIG. 5.—Graph showing development of amino nitrogen in Canada field pea and oat silage, 1918.

showed an increase in acidity closely resembling that of normal silage. The increase in the number of acid-producing organisms usually was followed by an increase in acidity.

The results of these investigations on the cause of formation of acid in silage agree with those of Lamb (*14*) and Hunter and Bushnell (*13*),

previously cited, in that the microorganisms are found responsible for the production of acid.

Plant enzyms evidently play an important part in the hydrolysis of protein, as is shown by the formation of ammonia after the silage had

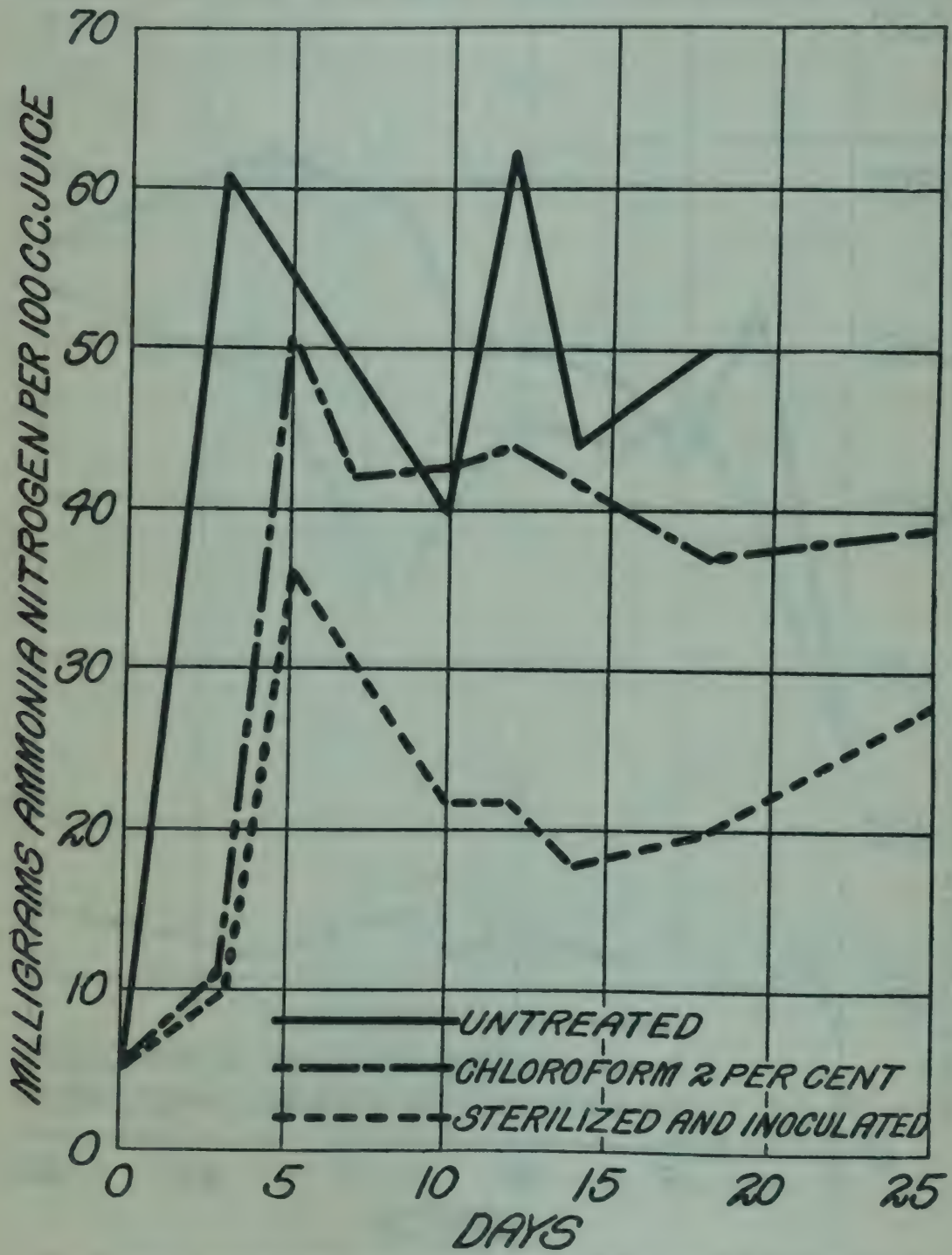


FIG. 6.—Graph showing increase in ammonia nitrogen in Canada field pea and oat silage, 1918.

been treated with 2 per cent chloroform, which inhibits the growth of bacteria. The formation of amino nitrogen in the chloroformed silage resembles that of normal silage. Practically no formation of amino nitrogen was observed in silage sterilized and inoculated. It is concluded that the hydrolysis of protein with formation of amino nitrogen

is due principally to the action of plant enzyms. Plant enzyms and microorganisms are both concerned in the formation of ammonia, the former being the more important.

The formation of ammonia occurred in silage treated with 2 per cent chloroform, which inhibits the growth of bacteria, and in silage sterilized and inoculated; but more ammonia was formed in the chloroformed silage than in that sterilized and inoculated. It will be noted that the increase in acid-producing organisms and the formation of ammonia are very well correlated. It is probable that the acid-producing organisms did attack the proteins, decomposing them with the formation of the ammonia. It has been shown by Bertrand and Weissweiller (*3*), Heineman and Hefferan (*10*), and Hastings, Evans, and Hart (*8*, *9*) that the lactic bacilli do have a digestive action on protein. Hart, Flint, and Evans (*7*), working on the action of certain bacteria in regard to the ripening of cheddar cheese, found that *Bacillus casei* had the ability to produce ammonia. Hunter (*12*) in an article on alfalfa silage suggests that a small part of the protein hydrolysis may be caused by the ability of acid-producing organisms to utilize protein as a source of energy in the absence of carbohydrates.

The largest number of yeasts were found in the first few days of the fermentation. They increased in number until all the free oxygen was consumed, then decreased. Lamb (*14*) states that alcohol is formed first by enzyms and later by yeasts. It is more easy to believe alcohol is formed first by yeasts and later by enzyms, because yeasts are present in greater numbers at the beginning of fermentation.

The only difference noted in the fermentation of Canada field pea and oat silage from that of corn silage was in the number of organisms belonging to the bulgaricus group. There were more organisms belonging to this group in corn silage than in silage composed of Canada field peas and oats.

No difference could be noted in the fermentation of corn silage when compared with corn and soybean silage.

CONCLUSIONS

(1) From the bacteriological and chemical analysis little difference can be noted between the fermentations taking place in silage composed of Canada field peas and oats, corn and soybeans, and corn only. There was a larger number of organisms belonging to the bulgarious group in corn silage than in the other types of silage studied.

(2) Production of acids was due to microorganisms.

(3) Yeasts apparently had little effect upon the fermentation of silage except during the first few days.

(4) Plant enzyms were chiefly responsible for the hydrolysis of protein with formation of amino nitrogen.

(5) The formation of ammonia was due to both enzyms and microorganisms.

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SANDY CRYSTALS IN ICE CREAM: THEIR SEPARATION AND IDENTIFICATION

By HARPER F. ZOLLER, *Chemist*, and OWEN E. WILLIAMS, *Dairy Manufacturing Specialist, Dairy Division, Bureau of Animal Industry, United States Department of Agriculture*[1]

Ever since Sato (*6*)[2] isolated crystals of lactose, sucrose, calcium phosphate, calcium citrate, etc., from the crystalline sediment occurring in sweetened condensed milk, there has been considerable speculation as to which of these substances are responsible for most of the sandiness found therein.[3]

The ice-cream industry utilizes large quantities of condensed milk, particularly evaporated skim milk and sweetened condensed whole milk. The tendency during the last few years toward building up the total solids of the ice-cream mix with milk solids other than milk fat has created a demand for the products mentioned. With their use there arose simultaneously the problem of "sandy" ice cream. Not infrequently wholesale quantities of ice cream develop this sandy or crystalline texture—sometimes large, hard crystals, sometimes fine and gritty, like starch. This wide variation in the size of the crystals caused much perplexity as to their composition in view of the work cited above on condensed milk. Since ordinary ice cream contains from 12 to 16 per cent of sucrose it was but natural that many should believe the sandiness, especially the large crystals, to be due to this substance; the finer might be due to any of the suspected substances.

In order to settle this question of "what constitutes sandiness" we took up the problem of isolating the "sandiness" after it had formed and of identifying it.

DEVELOPMENT OF SANDY CRYSTALS

A large quantity of a high solids-not-fat ice cream was prepared in a regular commercial power freezer. The composition of the mix was as follows: Fat, 10 per cent; sucrose, 14 per cent; milk solids not fat, 12 per cent. The raw products were ripened 40 per cent cream, evaporated skim milk, and pure cane sugar. The mix was frozen in the regular time with brine at about 10° F. (— 12.22° C.). It was immediately packed, after freezing, in 2-quart tinned cans and placed in the hardening

[1] The authors express their indebtedness to G. L. Keenan and E. T. Wherry, of the Bureau of Chemistry, to the former for his courtesy in preparing the photomicrographs and to the latter for checking the crystallographic features of lactose.

[2] Reference is made by number (italic) to "Literature cited," p. 795.

[3] It would be unfair to create the impression that Sato actually isolated sucrose crystals from all samples of condensed milk. In fact, the sucrose crystals were isolated only rarely from condensed milk, and in those cases he specifically states that they were condensed to an unusually low water content.

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box. This box was insulated with wood and held a hardening mixture of ice and rock salt. The ice cream was repacked each day in this box.

On the fourth day very fine sandy crystals appeared. The crystals were developed by allowing the cans to become "heat shocked" (by setting them in contact with the air of the room for about 20 minutes) every other day after the sandiness first became perceptible.

The dimensions of the crystals were observed frequently through the low-power objective of a compound microscope. When the sandiness first became perceptible to the tongue and between the fingers, the crystals were clearly visible in the microscope. The general form of the crystals remained the same throughout 20 days' observation, although they grew much in size during this interval.

When the crystals had grown to a sufficient size they were separated from the parent mass. Plate 137 illustrates the appearance of the crystals in the frozen ice cream described above, when a drop of the ice cream was placed upon a glass slide and then viewed through the microscope in polarized light. The crystals showed plainly the natural maize-shaped form of lactose hydrate.

SEPARATION OF THE CRYSTALS

The sandy crystals were separated from several different preparations of ice cream by the following method: Frozen cream was allowed to melt at the temperature of the laboratory, about 25° C. When completely melted it was poured into large centrifugal tubes of 100 cc. capacity and centrifuged at 2,000 revolutions per minute for 10 minutes. The fat and liquid layers were poured from the sediment; the liquid portion was now entirely free from sandiness, the latter having been thrown down in the form of sediment. A drop of the sediment examined on the microscopic slide seemed to be made up entirely of crystals of one form—some large and some small.

Before the crystals were proved to consist entirely of pure lactose they were isolated from the sediment for the purpose of holding any sucrose or lactose crystals in the crystalline form. This was effected by saturating water with all the sucrose and lactose that it could hold at 2° C. It was then warmed to 5°, and the sediment in each tube was thoroughly shaken with about 75 cc. of it. Then the tubes were again centrifuged. This operation was repeated with the addition of 50 per cent acetone to the washing fluid. The product was further freed from liquid on a Büchner funnel.

The crystals were obtained in a pure white condition. More than ½ kilo was obtained in this manner. They were dried to constant weight on filter paper in a boiling-water oven. Plate 138, A, illustrates the appearance of these isolated crystals. They show plainly the effect of the washing mixtures but retain their characteristic maizelike or wedge-like form.

IDENTIFICATION OF THE CRYSTALS AS LACTOSE HYDRATE

MICROSCOPICAL EXAMINATION

The ease with which milk sugar usually crystallizes and the very characteristic form of its crystals suggest the use of the microscope as the most accurate means of identifying it. As mentioned in the earlier part of the text it is possible to detect and follow microscopically the growth of the crystals in the ice cream.

For the purpose of identification of the sandy crystals it was necessary to have at hand pure specimens of lactose and sucrose crystals. Rather than accept the market lactose as standard for recrystallization a quantity of pure lactose was obtained by evaporating some milk whey from cheese-making in a vacuum pan to a concentration of 8 parts by weight of whey to 1 part by weight of product. The resulting lactose was filtered and purified according to the customary methods.

The crystalline lactose thus obtained was the α form, or commonly recognized lactose hydrate ($C_{12}H_{22}O_{11}.H_2O$). According to Groth (*1, p. 450*) its crystallographic features are—

Monoclinic-sphenoidal. Cleavage in three directions nearly at right angles. Refractive indices, $\alpha = 1.517$; $\beta = 1.542$; $\gamma = 1.550 \pm 0.005$ $Bx^a \wedge c = 10°$, $\wedge a = 99°$. $2E = 33\text{-}1/2°$. Sign $-$, sp. gr. $1.525 - 1.534$.

Some of these crystals were placed upon a microscopic slide, and a photomicrograph was obtained with polarized light. The photograph exhibits the characteristic tomahawk-shaped crystals which are always presented by α-lactose when the crystals are allowed to develop spontaneously below 93° C. (*3*). When they crystallize at 93°, or a little above, lenticular needles appear (γ or anhydride form) (*2*), which are markedly different to the eye. These gradually transform into the tomahawk type as the temperature drops below 93°. The photomicrograph of these lactose-hydrate crystals is shown in Plate 138, B.

The comparison of these pure lactose crystals with those within the ice cream in Plate 137, as well as with that of the sandy crystals separated in a pure condition from the same ice cream, shown in Plate 138, A, shows the sandy crystals to be identical in form with those of pure lactose. It is but natural that we should realize the effects shown in Plates 137 and 138, A, of substances tending to hinder perfect growth of the crystals. Also the solvent action of water is evident in the partially dissolved facets and less sharp outlines. Otherwise there is no difference in the appearance between the crystals in Plate 138, B, and those in the first three figures.

A fully developed crystal of sucrose is shown in Plate 138, C. The sucrose crystals were grown within a pectin gel from a supersaturated sucrose solution (500 per cent solution) at ordinary temperature. There is not the least resemblance between this crystal and those isolated from ice cream.

CHEMICAL ANALYSIS

Some of the isolated sandy crystals mentioned in the section on separation, and similar to those photographed in Plate 138, A, were analyzed for lactose, without inversion, according to the Munson and Walker method,[1] described by Leach (*4, p. 598*). The average of three determinations gave 99.86 per cent calculated as lactose hydrate from Munson and Walker's tables. It is evident that the quantity of sucrose appearing as sandy crystals is insignificant.

DISCUSSION

The results of our experiments upon a number of sandy ice creams lead us to conclude definitely that the sandiness is due to the crystallization of lactose from a supersaturated solution.

The suspicion, fostered by a number of persons in the dairy industry, that sucrose is responsible for this sandiness is not founded on scientific principles. The solubility, recorded by many investigations, of lactose in water at 10° C. is about 17 gm. per 100 gm. of water. The solubility of sucrose at 12° is given as 198.6 gm. per 100 gm. of water. When these solutions are cooled they become supersaturated, provided they contain the above-mentioned quantities of the respective sugars. In sucrose solutions, however, we meet with the experience of the sugar technician, Prinsen Geerligs (*5, p. 301*), who tells us that sucrose solutions frequently act like glue at low temperatures and can be supercooled to a state in which they can not be poured. In the highly supersaturated, supercooled condition it is very difficult to induce them to crystallize. When accomplished it requires weeks and even months for the crystals to develop in size, particularly if much colloidal material, such as gum, pectin, or protein, is present in the solution. It required several months for the perfect crystal of sucrose shown in Plate 138, C, to develop even to this size.

In the case of lactose, because of its low solubility in water, compared to sucrose, it is more difficult to form supersaturated solutions of any great magnitude. When its solutions are concentrated it crystallizes without much excitation. Indeed, it will persist in crystallizing even in the presence of complex protective substances which check crystallization of many other compounds.

An ice-cream mix of 10 per cent fat, 12 per cent milk solids not fat, and 14 per cent sucrose contains, if we assume that the two sugars compete for only the water in the mix, about 22 per cent sucrose solution and about 10.5 per cent lactose solution. When we recall the fact that sucrose is about 10 times as soluble as lactose in water, we readily see that the physical conditions existing in frozen ice cream are unsuited to the crystallization of sucrose.

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SUMMARY

(1) The "sandiness" in ice cream has been isolated and identified as lactose.

(2) The growth of the "sand" crystals can be followed satisfactorily with the microscope.

(3) The form of lactose appearing in the ice cream is the normal α crystal which crystallizes from water solutions in tomahawk-shaped prisms and from protein solutions in more rugged or maize-shaped crystals.

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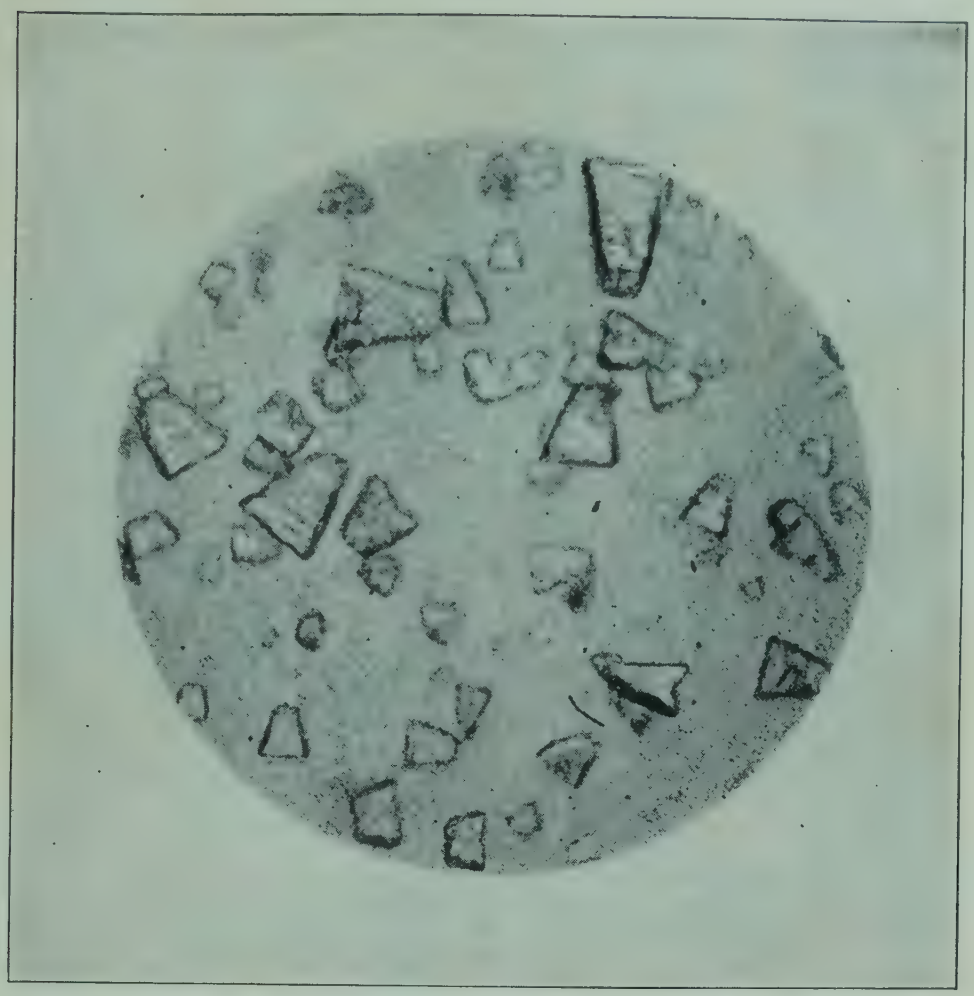
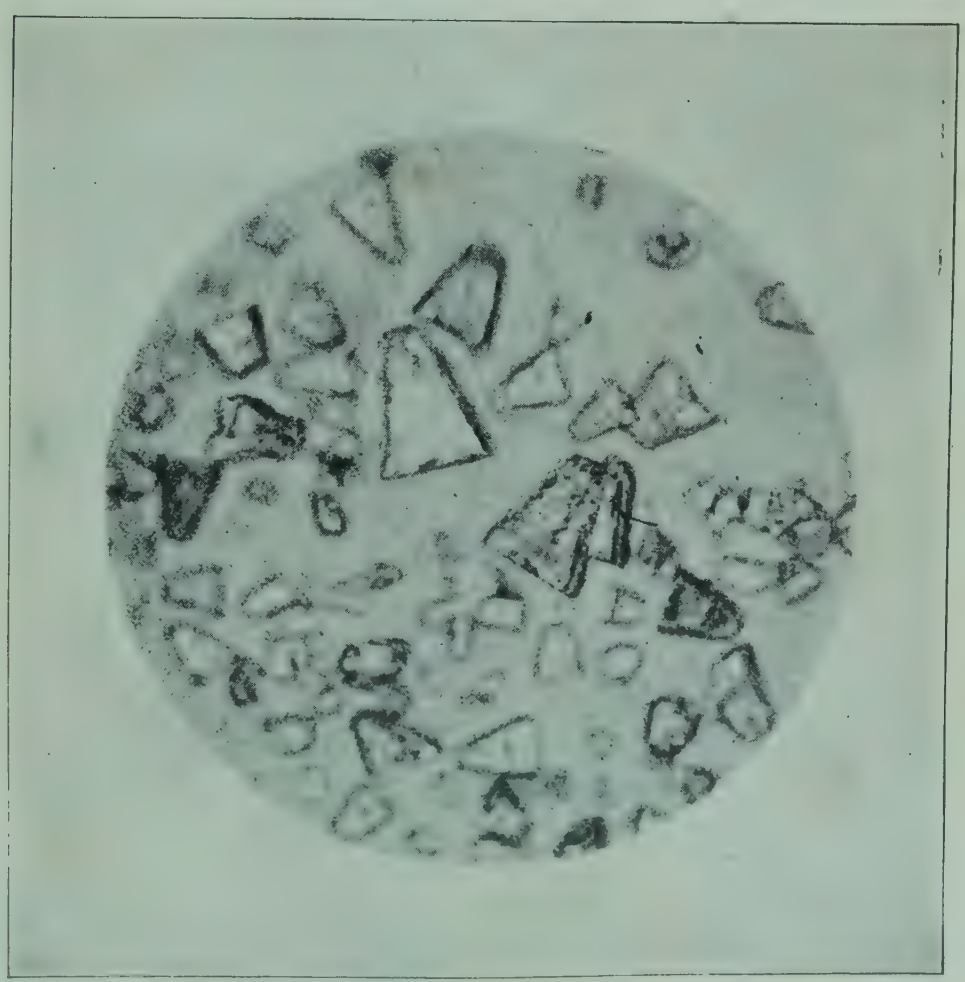
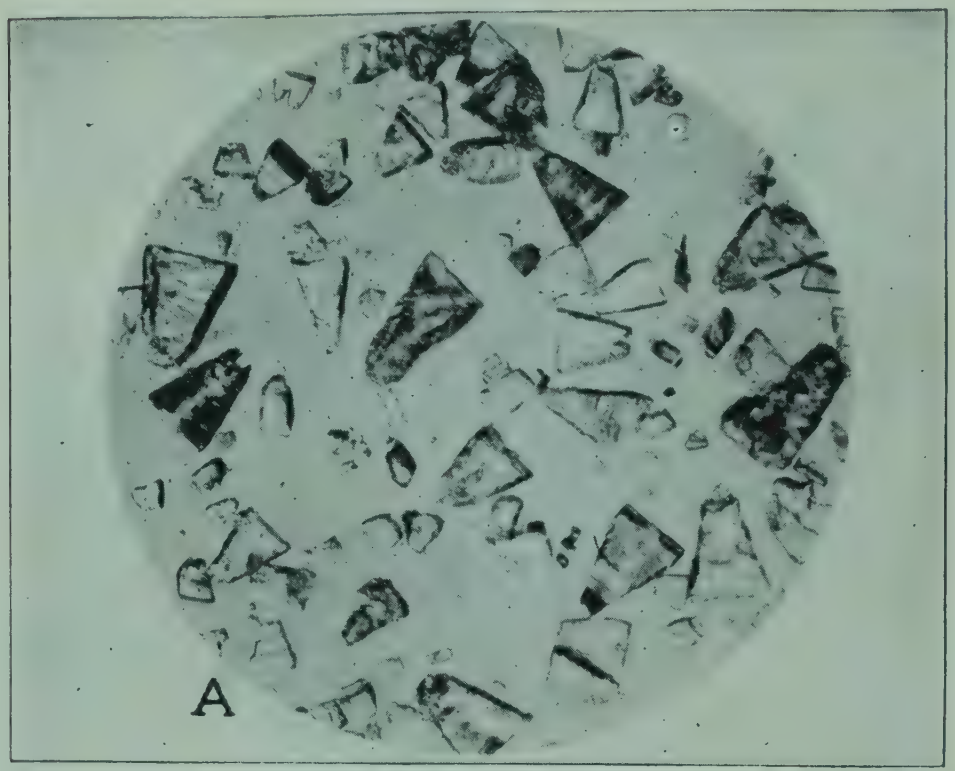
PLATE 137

Photomicrographs of the "sand" crystals in ice cream, made by placing a drop of the melted ice cream upon a microscopic slide and covering it with a cover glass. This was then placed upon the stage of the photographic microscope and photographed with polarized light. × 90.



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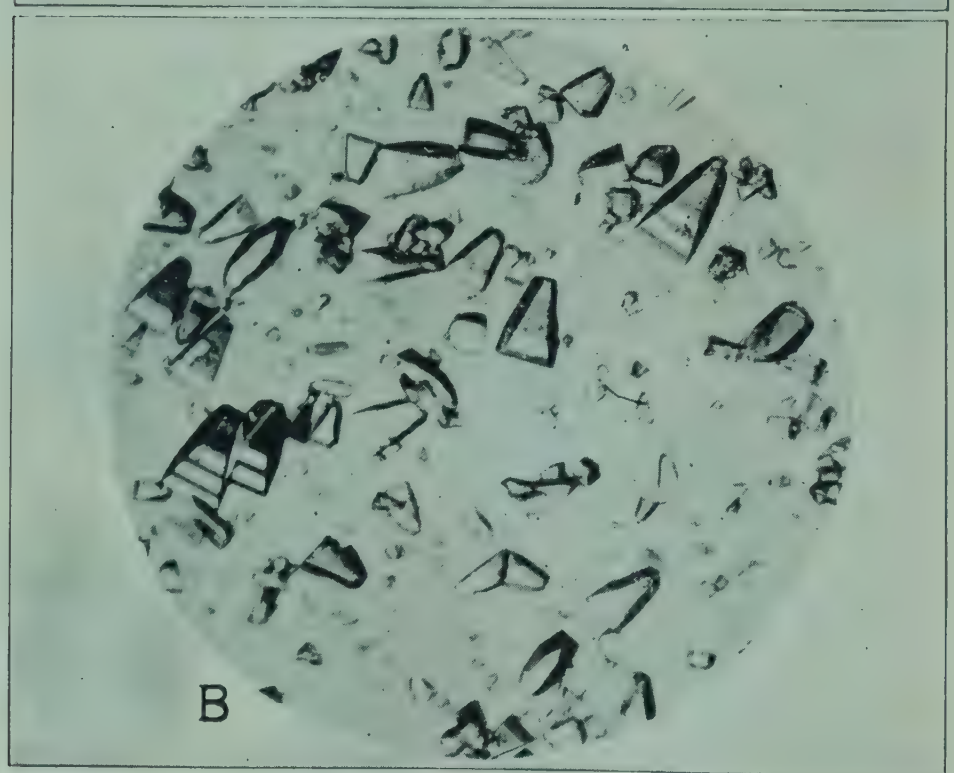
B

C

Plate 138

A.—Photomicrograph of the isolated "sand" crystals from some of the same ice cream used for Plate 137. Photographed with polarized light. × 90.

B.—Photomicrograph of some pure recrystallized lactose hydrate from condensed whey. Photographed with polarized light. × 90.

C.—Photomicrograph of a crystal of pure sucrose. Photographed in plane light. × 5.

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SOLENOPOTES CAPILLATUS, A SUCKING LOUSE OF CATTLE NOT HERETOFORE KNOWN IN THE UNITED STATES

By F. C. BISHOPP, *Entomologist, Investigations of Insects Affecting the Health of Animals, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

In 1904 Enderlein[1] described a single male sucking louse taken on cattle in Leipsic, Germany, as a new genus and species, *Solenopotes capillatus*. Apparently no further reference was made to this insect until 1916, when Ferris[2] incidentally mentioned the species, stating that he considered the Enderlein type specimen as being immature and concluding that the species was a synonym of *Linognathus vituli* L.

In examining the specimens of Anoplura in the collection of the Dallas laboratory of the Bureau of Entomology in 1917, H. P. Wood noted that some of the material collected on cattle did not appear to be either *Linognathus vituli* L. or *Haematopinus eurysternus* Nitzsche. This led to a search of literature for other species recorded from this host, and the references mentioned above were found. Upon comparing the material in question with Enderlein's description it was evident that it was *Solenopotes capillatus*.

Material in the collection at the Dallas laboratory indicates that the species has a wide distribution in the United States, and notes upon it show that at times it may become a serious cattle pest. Data on the collections are as follows:

Bishopp No. 9192, Dallas, Tex., March 20, 1910, many specimens on bull (*Bos taurus* L.) in laboratory yard, F. C. Bishopp, collector; Bishopp No. 2681, Viewpoint, Oreg., March 8, 1913, heavy infestation of range cattle (*B. taurus*), H. H. Hatch, collector; No. 4292, Riverside, Md., February 14, 1915, on calves, F. C. Bishopp, collector; No. 7485, Dallas, Tex., November 13, 1917, many specimens on cow in laboratory yard, H. P. Wood, collector; No. 8487, Olympia, Wash., December 28, 1918, on Jersey bull, R. W. Wells, collector; No. 8500, Uvalde, Tex., January 10, 1919, on calf, D. C. Parman, collector; No. 8995, Bennington, Vt., March 17, 1919, on cow, R. W. Wells, collector; No. 9699, Herkimer, N. Y., March 20, 1920, on calf, F. C. Bishopp, collector.

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[2] FERRIS, G. F. CERVOPHTHIRIUS CRASSICORNIS. (N.) (ANOPLURA). *In* Ent. News, v. 27, no. 5, p. 197–200. 1916.

The species shows a marked tendency toward attaching in dense groups about the head and neck of the host. Sometimes these patches are almost circular.

In 1917 H. P. Wood made an effort to secure oviposition of the lice under control on cattle, but with little success. Evidence was gained which indicates that the incubation period is rather long, probably about 12 days, and also that the eggs will not hatch when removed from the host.

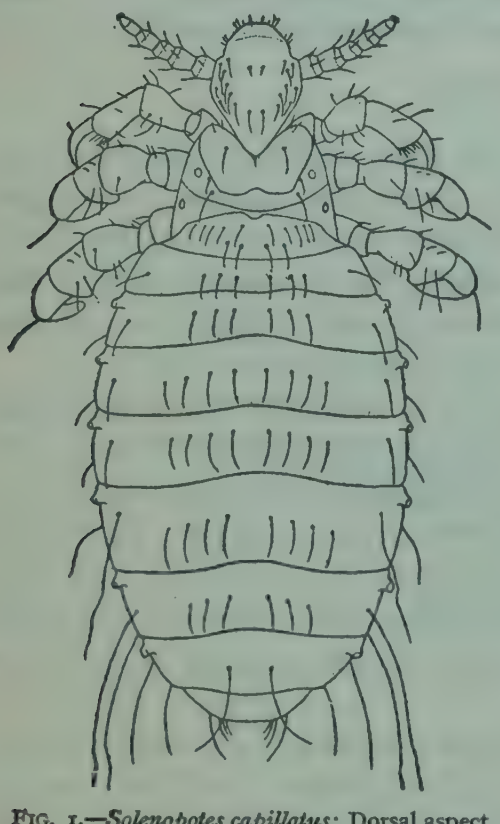

FIG. 1.—*Solenopotes capillatus:* Dorsal aspect of female. × 40.

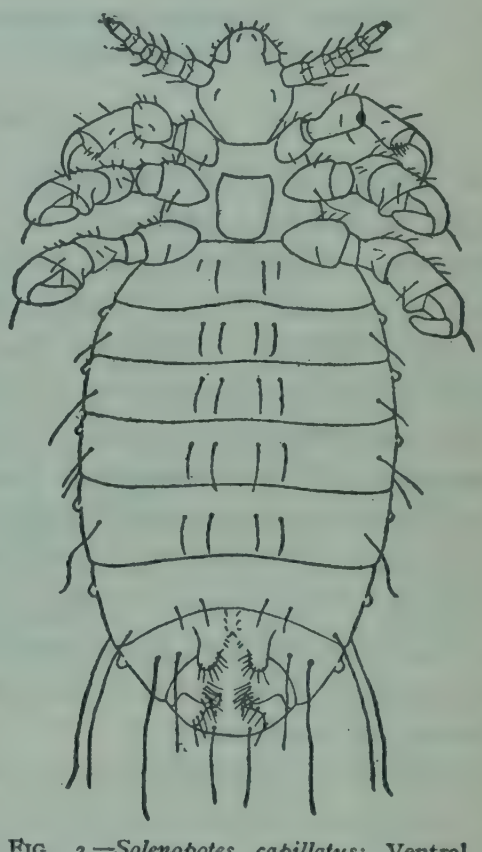

FIG. 2.—*Solenopotes capillatus:* Ventral aspect of female. × 40.

DESCRIPTION

THE FEMALE

As the female of this species has not been described, it will be characterized fully here.

Length (fig. 1 and 2) about 1.5 mm., greatest width about 0.7 mm., thorax and legs brownish yellow, abdomen grayish blue.

HEAD.—Length 287 μ, width 215 μ. Anterior margin rather broadly rounded, lateral margins slightly widest behind antennæ, somewhat narrowed at transverse groove just anterior to antennæ, posteriorly pointed. Antennæ attached about one-third length of head from anterior border, rostrum just ventrad of anterior margin, a chitinized rim around anterior margin above rostrum. Antennæ slightly longer than greatest width of head, basal segment markedly broader than succeeding segments, segments 3 and 4 somewhat shorter than others. Several short spines on anterior margin of head, two spines on ventral surface behind antennæ, dorsally with a slightly curved row of about five spines on either side, one somewhat smaller spine outside the anterior end of this row just in front of the antenna on either side, and two near the margin behind the antennæ. Near the median line is a small spine on either

side opposite the point of attachment of the antennæ and toward the posterior margin of the head another pair, somewhat longer than any others, about the same distance from the median line

THORAX.—About 200 μ long by 344 μ wide, deeply incised anteriorly to receive the head, narrowed somewhat anteriorly and with rounded sides. Division of thorax fairly distinct dorsally and the mesothorax and metathorax bearing at the margin a rather prominent but not projecting spiracle. Prothorax with two rather strong submedian spines and mesothorax with two or three small and one strong spine. Sternal plate about 172 μ long by 114 μ wide, not very highly chitinized, slightly narrow posteriorly, anterior end truncate, posterior end rounded.

LEGS.—Rather short and stout, anterior pair much shorter, more slender, and less chitinized than others. They terminate in long, slender claws, while the posterior legs are provided with highly chitinized dark reddish brown terminal segments and blunt claws. A few short hairs on all leg segments.

ABDOMEN.—Elongate oval, narrowing gradually to terminal segment which is broadly rounded; abdomen dorsally with a considerable number of yellowish, rather slender spines distributed as follows: First segment, two transverse rows, anterior with 8 or 10 spines irregular in length, posterior with from 4 to 6; second, from 4 to 6; third, 6; fourth, from 6 to 8; fifth, from 6 to 8; sixth, 8; seventh, 6; eighth, 2; the last rather longer than the others. Near the lateral margin of the second to seventh segments is a single spine on each side, those on the sixth and seventh being two or three times as long as the others and hairlike toward their tips. On the venter the spines are less variable in number than on the dorsum, the second to eighth segments with 4 submedian spines, the outer pair on second segment being small and those on the eighth segment being farther removed from the median line and very long and hairlike at tips. Near the lateral border on either side is a single row of spines on the third to seventh segments, those on the sixth and seventh segments being long (longer on the seventh) and hairlike toward the tips.

FIG. 3.—*Solenopotes capillatus*: Eighth and terminal segments of abdomen of female. × 53⅓.

On the posterior margin of the eighth segment are two genital flaps or protuberances margined with 7 rather stout spines (fig. 3). Between these on either side are 2 minute spines, and from the middle to the anterior border of this segment near the median line are 3 pairs of very short spines. The chitinized bands on the terminal segments are rather narrow dorsally and broad on their ventral margins. There is a slight projection on this band in the internal anterior angles which bears 3 or 4 stout spines on each side near the margin. Just anteriorly from the inner angles of the bands occur on each side a group of about 6 moderately stout spines, and mesad from their borders about 3 small spines.

The second to eighth segments are provided near their anterior margin with projecting, rather large spiracles 29 μ in diameter.

THE MALE

While Enderlein's [1] description of the male is fairly complete, it is deemed advisable to redescribe this sex and point out some of the variations in the distribution of the abdominal spines.

Length 1.2 mm., width 0.6 mm.

HEAD.—Length 287 μ, width 208 μ. Rather broadly rounded anteriorly (fig. 4), widest just behind antennae which are attached about one-fourth of the length of the

[1] ENDERLEIN, Günther. op. cit.

head from the anterior margin. A row of about 6 small spines extending across near the anterior margin dorsally and a longitudinal row on each side with about 6 spines. One short spine in front and 2 behind each antenna and also 2 large spines toward posterior edge of head medially. Ventrally a spine of moderate length on each side behind and mesad of base of antennæ. Mouth parts slightly ventrad of anterior extremity, a chitinized transverse ridge running across the anterior margin just above the mouthparts.

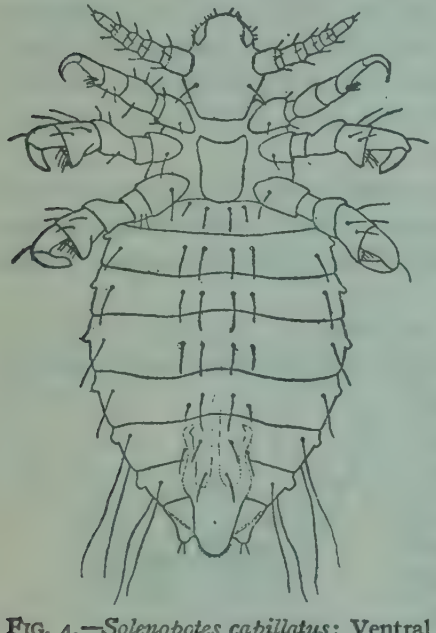

FIG. 4.—*Solenopotes capillatus*: Ventral aspect of male. × 40.

THORAX.—Much as in female, a few short and one long spine on either side of pronotum and mesonotum. Spiracles on pronotum and mesonotum prominent. Sternal plate more elongate than in female and slightly concave on lateral border.

ABDOMEN.—Greatest width at about fourth segment, elongate oval. Second to eighth segments provided with projecting spiracles as in female, ninth segment with a distinct notch on either side, the central terminal portion being rather stout and bearing a number of small spines along the border. On the dorsal side segments armed submedially as follows: The first segment with two transverse rows, anteriorly to these 6 small spines and posteriorly about 8 spines, the two median being the largest; second segment, 8 spines; third, 6 spines; fourth, 8 or 10 spines; fifth, 8 spines; sixth, 8 spines; seventh, 6 or 8 spines; eighth, from 4 to 6 spines. Near lateral margins another row of spines, one spine to each segment, those on seventh and eighth segments being very long and hairlike, those on first segment small. Ventrally, the second to seventh segments with a row of 4 spines submedially and the eighth with 2 spines. All ventral spines somewhat stronger than the dorsal ones. Near the lateral margin the second to eighth segments provided with a single spine on either side, those on seventh and eighth segments being long and hairlike, as on the dorsal surface.

Genital frame (fig. 5) rather simple, apparently consisting of six parts. Basal portion forked for about one-fifth of its length at the posterior end where it articulates with two elongate plates which in turn join at their posterior end with two other plates of slightly greater length. These diverge posteriorly and bend dorsally and cross the anterior ends of a wishbonelike plate the posterior end of which terminates in a rather acute, highly chitinized point known to some as the pseudopenis. The penis is simple, nearly equaling in length the inclosure of the frame, the aperture situated well back between the legs of the wishbonelike plate.

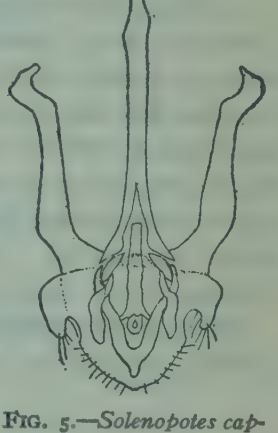

FIG. 5.—*Solenopotes capillatus:* Genital frame. × 106⅔.

NYMPHAL STAGES

First instar, color pale yellowish before feeding, except for parts of abdomen which are darker; after feeding, much as in adult though somewhat paler; tarsi and claws yellowish to light brown. Later instars darker.

HEAD.—Showing slightly stronger transverse groove than in adult.

THORAX.—About as in adult, but slightly less chitinized.

ABDOMEN.—In first instar very elongate, not wider than thorax; in later instar markedly broader, a shallow submarginal groove on each side running from first segment to about the seventh, where they join. Along the line of this groove on each side is a row of depressions, one near the anterior border of each segment. Spines about as in adult female though hairs are very short except on the sides of seventh and eighth segments on which they extend well beyond the tip of the abdomen. Apex of abdomen slightly bilobed, spiracles projecting distinctly on the third to eighth segments, inclusive.

THE EGG

Color pale yellowish. Elongate oval, the side next to the hair upon which attached practically straight, the other side curved, set at a comparatively small angle from the hair and cemented to it by a basal, somewhat heart-shaped, broad and comparatively short attachment (fig. 6). Length of egg, not including attachment, 735 μ, greatest width 278 μ at right angles to a plane passing through the egg and hair to which it is attached, the other diameter being about 244 μ. The well-defined, somewhat elevated operculum set almost at right angles to the straight side of the egg; diameter about 190 μ; its height about one-third of its diameter; length of attachment clamp about 190 μ, its greatest width about 180 μ. Surface of egg minutely reticulated, under moderate magnification giving a granular appearance. Operculum with a number of slight protuberances which give it a deeply roughened appearance.

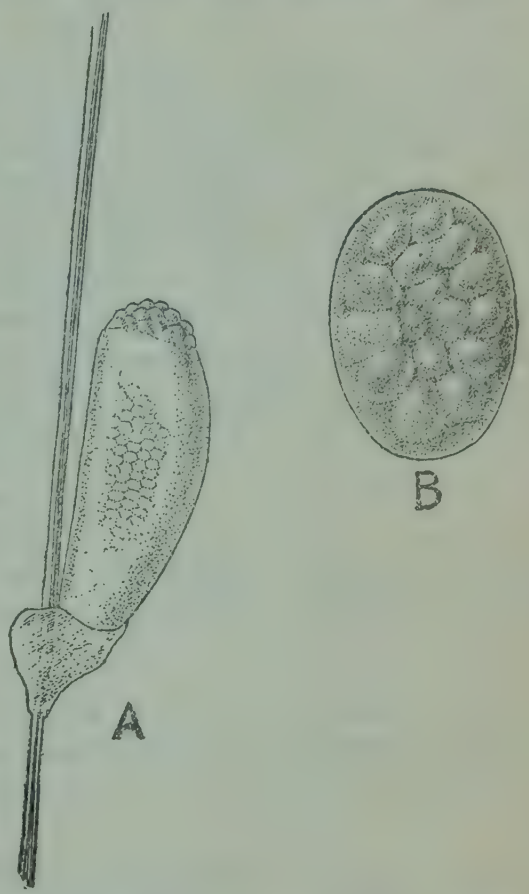

FIG. 6.—*Solenopotes capillatus:* A, Egg attached to hair, × 40; B, operculum of egg, greatly enlarged.

A number of eggs are frequently attached to a single hair and may be found anywhere from the base to the tip of the hair.

RATE OF CULM FORMATION IN BROMUS INERMIS

By L. R. WALDRON
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INTRODUCTION

Various investigators have made growth studies upon different organisms. Working with the rat, Donaldson[1] and other workers have published a number of formulas showing the growth of certain organs with respect mainly to the growth in body weight. The curves are mostly logarithmic in type. They are based on careful data and show the general trend of organic development relative to size of animal.

Pearl has made studies in growth phenomena and has developed curves to express the general type of growth. Working with Ceratophyllum,[2] he developed a logarithmic curve of the general type

$$Y = A + C \log (x - a)$$

which furnished a symbolical expression of the manner of growth dealing with rate of increase of leaves per whorl along the axis of the plant. He says:

> The mean number of leaves per whorl increases with each successive whorl, and in such a way that not only does the absolute increment diminish, but also the rate of increase diminishes as the ordinal number of the whorl measured from a fixed point increases.

In other words, this phase of growth is rather rapid at first and then slows down, both absolutely and comparatively.

Working with maize, Pearl and Surface[3] secured data upon height of plant which, from their previous studies, they were confident could be fitted with a logarithmic curve, probably of the type

$$Y = A + B \log x.$$

Here again the growth movement is rapid at first, but this rapidity decreases later. Pearl goes so far as to state that the growth of an organism can usually, if not always, be described by a curve of this type.

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[1] DONALDSON, Henry H. THE RAT. REFERENCE TABLES AND DATA FOR THE ALBINO RAT (MUS NORVEGICUS ALBINUS) AND THE NORWAY RAT (MUS NORVEGICUS). 278 p., 31 charts, 89 tab. Philadelphia, 1915. References to the literature, p. 214-267, and references at end of chapters. Mem. Wistar Inst. Anat. and Biol., no. 6.

[2] PEARL, Raymond. VARIATION AND DIFFERENTIATION IN CERATOPHYLLUM. 136 p., 26 fig., 2 pl. Washington, D. C., 1907. Literature cited, p. 135-136. Carnegie Inst. Wash., Pub. 58.

[3] PEARL, Raymond, and SURFACE, Frank M. GROWTH AND VARIATION IN MAIZE. *In* Ztschr. Indukt. Abstam. u. Vererb., Bd. 14, Heft 3/4, p. 97-203, 15 fig. 1915. Bibliography, p. 171-172. Preliminary abstract *in* Proc. Nat. Acad. Sci., v. 1, no. 4, p. 222-226.

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In the work at hand, instead of a rapid increase in the early stages and then a falling off in size of increments, growth comes about by constantly increasing increments. It did not appear in the present study that the data were amenable to a logarithmic type of curve.

Blackman [1] has applied the compound interest law, commonly used by physicists, to plant growth, particularly to changes in weight undergone by annual plants from the germination stage to more mature stages. A fuller discussion of this law will be undertaken when the data have been presented.

CULM FORMATION IN BROMUS INERMIS

In the spring of 1916 a large number of seedlings of *Bromus inermis* Leyss were started in flats and later planted out in the field as individual plants 3.5 feet apart each way. The seed came from a Canadian source and was commercial seed. It had not been pedigreed or selected to any type. On June 19 countings were begun of the number of culms per plant of a limited number of plants.[2] At the time of first counting the plants had a height of 92.2 mm. as an average of the longest culm per plant, measuring to the extreme tip of the leaf. Stooling had begun on most of the plants. This consists essentially, in its earlier phases, in the formation and growth of additional culms springing from, at, or near the base of the primary culm. This study is concerned primarily with the rate of production of such secondary culms and the relation of their number to other characters of the plant.

Countings were made every five days except for the intervention of Sundays and holidays. The dates of the countings were June 19, 24, 29; July 5, 10, 15, 20, 25, 31; August 5, 10, 15, 22, 26, 31; September 5, 11.

There were thus a total of 17 countings, embracing a period of 85 days, including the dates of the first and last countings. At the time of the first counting the number of culms per plant [3] averaged 2.37, with a minimum of 1 and a maximum of 5. It is seen that at the first count growth was still in its earlier stages, from the standpoint of this study. At the date of the last count, September 11, culm formation was still in progress and apparently unaffected by weather conditions. The countings were stopped at this time, primarily because the large number of culms per plant made a precise count difficult.

The countings were continued over an interval of time sufficiently long so that the law of their rate of formation during favorable growth conditions can be deduced with a reasonable degree of satisfaction. A study of the rate of cessation of formation during the close of the growing

[1] BLACKMAN, V. H. THE COMPOUND INTEREST LAW AND PLANT GROWTH. *In* Ann. Bot., v. 33, no. 131, p. 353–360. 1919.

[2] Data were secured on 97 plants for the period of study.

[3] A culm was recognized as such when it had attained an extreme height of 25 mm., measurement being made from the tops of stakes driven down nearly flush with the surface of the ground.

season would be of interest but evidently of lesser importance than the problem at hand. In the one case the rate of formation of a turf is dealt with, while in the other case merely a minor phase of the problem would be studied, one relating to the behavior of the plant at the close of the growing season.

The raw data are not presented because of limited space. In Table I are presented the means, differences of the means, medians, and ranges for each date. The standard deviations, and consequently the coefficients of variation, were not calculated, as it was not seen that they would serve any useful purpose. It is obvious that the standard deviations and likewise the variations increase with great rapidity as the season progresses. Some of the plants stool with much greater rapidity than others, with the result that distances between the extreme limits constantly increase.

The means and medians were calculated by arithmetical methods. The data were almost necessarily left ungrouped, as the class units suitable for the earlier dates would be quite unsuitable for the later dates.

TABLE I.—*Means, medians, and extreme ranges of number of culms per plant of Bromus inermis at various dates*

Date.	Mean number of culms.	Difference of means.	Median.	Range.
June 19	2.37	.....	2.35	1 to 5
24	2.99	0.62	3.05	1 to 7
29	5.07	2.08	5.03	1 to 11
July 5	9.39	4.32	8.94	1 to 19
10	11.16	1.77	10.75	4 to 22
15	13.45	2.29	13.40	6 to 35
20	16.05	2.60	16.00	6 to 41
25	21.86	5.81	21.75	7 to 46
31	30.52	8.66	29.75	10 to 63
Aug. 5	35.11	4.59	34.38	12 to 78
10	43.55	8.44	42.75	17 to 107
15	53.72	10.77	51.25	21 to 133
22	68.46	14.72	65.00	24 to 171
26	77.74	9.28	73.00	24 to 214
31	89.57	11.83	82.75	32 to 254
Sept. 5	101.79	12.22	95.33	39 to 293
11	117.77	15.98	106.50	40 to 329

ANALYSIS OF TABLE I

From Table I one notes that the number of culms per plant increases with more or less irregularity from 2.37 at the time of the first counting to 117.77 on September 11, the date of the final count. This irregular advance may be ascribed in part to irregularities in moisture and temperature but to a considerable extent to the occasional variation of dates in counting. When the means are plotted, the irregularity due to the last-

mentioned cause may be made to disappear by arranging the abscissas in accordance with dates of counting.

For all countings except June 24 the median is smaller than the mean, and in these cases the skewness is presumably positive. Positive skewness may perhaps usually be presumed in cases of this kind. A certain number of plants progress slowly, while the values of the remainder are scattered over a wide range, but all in advance of the earlier stages. This is indicated by a study of the extreme ranges. The differences between the minimum values are much less than the corresponding differences between the maximum values.

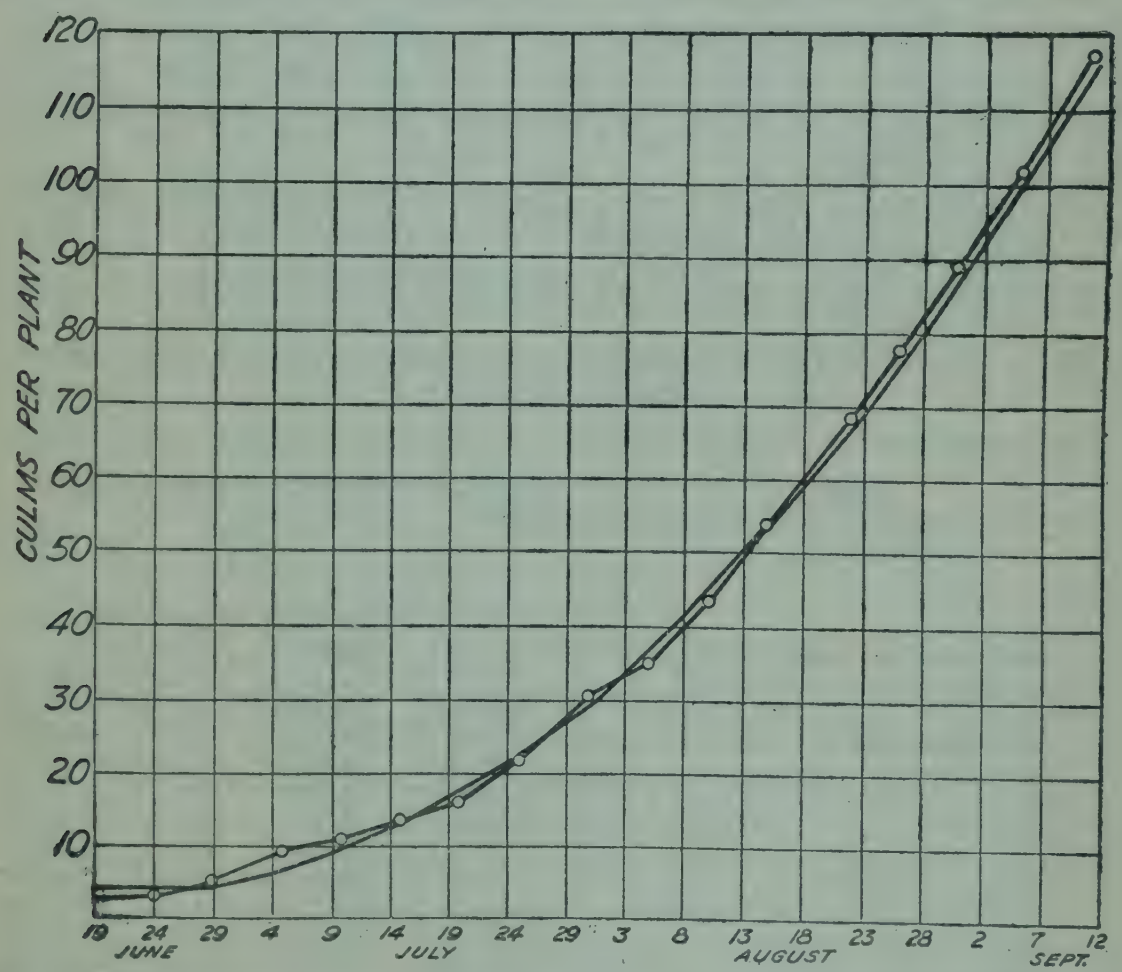


FIG. 1.—Plotting of average number of culms per plant at different dates of counting and curve fitted to the observations.

More interest centers about the discussion of the mean number of culms taken seriatim. To facilitate discussion these means have been plotted and are shown in figure 1 as the solid line. The abscissas in the figure for the observations are placed in accordance with the dates of counting, which are usually five days apart. The plotted observations show, on the whole, a very smooth series. From an inspection of the graph there is evidently something of a spurt in culm formation around July 1 and also a marked falling off around August 5. There were no obvious weather changes to account for these comparatively slight modifications from what one might consider the normal course of events.

DEVELOPMENT OF EQUATIONS

In order to give this series of observations a symbolical expression it will be necessary to develop an equation which, when plotted, will show a reasonable accordance with the observations. Such an equation was developed according to the method of moments developed by Pearson.[1] In the problem at hand there were 17, counting dates, and this gives us at once the odd number of classes required when this method is used. In working out the problem the first time the various counting dates were considered as natural numbers up to 17. The range would therefore be 1 to 17, or, in terms of the solution,

$$2l = 16, \text{ or } l = 8.$$

By this method of working the problem it is seen that the dates of counting are spaced at equal distances, when as a matter of fact occasionally 6 days (one instance each of 4 and 7 days) elapsed between two consecutive counting dates. To attempt a correction of this error the range was made coextensive with the entire counting period. The origin of x was taken at the middle counting date and extended 42 days both plus and minus. The table of corrective terms for moments of trapezia given by Pearson [1] had to be extended for values of L_1 and L_2, as l equaled 42 in this instance. This was easily done. Working the problem according to the first or non-weighted method, the following equation of a parabola of the second order was developed:

$$\text{(I)} \quad y = 40.313 \left\{ 0.749029 + 1.405701 \left(\frac{x}{l}\right) + 0.752914 \left(\frac{x}{l}\right)^2 \right\}.$$

The equation of a similar parabola developed according to weighted method was

$$\text{(II)} \quad y = 40.313 \left\{ 0.749786 + 1.395915 \left(\frac{x}{l}\right) + 0.750641 \left(\frac{x}{l}\right)^2 \right\}.$$

An attempt was made to fit parabolas of the third and fourth order by the non-weighted method, but with poor results. No attempts were made to fit parabolas of higher orders than the fourth.

Equations I and II are seen to be very similar, and both seem to give quite satisfactory fittings, equation II being perhaps appreciably better. There was a doubtful possibility that a better fit could be secured if a parabola of the third order, for instance, had been obtained by the method of weighting, but to secure this was not possible with the calculating machine at hand.

Equation II is fitted in figure 1 to the observations as shown by the curve with circles. Except for the two lower and four higher values of

[1] PEARSON, Karl. ON THE SYSTEMATIC FITTING OF CURVES TO OBSERVATIONS AND MEASUREMENTS. *In* Biometrika, v. 1, pt. 3, p. 265-303; v. 2, pt. 1, p. 1-23, 10 fig. 1902.

x this equation is appreciably better than equation I. However, for purposes of extrapolation beyond the upper range of observations, equation II may be considered the more conservative, as shown by the calculated results. The parabola obtained from equation I is very close indeed to the observations for the upper limits of the range, and perhaps this equation would give more accurate results for extrapolation purposes when calculations are made beyond the range. The extrapolated values would be the greater for equation I.

APPLICATION OF EQUATIONS

Assuming that either equation I or II is sufficiently correct for practical purposes, one may say that each represents or formulates the law of the rapidity of culm formation in *Bromus inermis* under the conditions indicated. These are: (1) Culm formation during the first or seedling year; (2) free from competition; and (3) under favorable soil and moisture conditions. The equations could, no doubt, be applied to certain other perennial grasses of similar growth with only slight modifications. Certainly they would need to be modified somewhat when applied to *Bromus inermis* grown under conditions other than those cited in this study.

These equations serve the purpose of indicating, at some future period for the plants under study, the amount of growth in number of culms produced, with all conditions remaining constant. It is true enough that conditions would not remain constant for any considerable future period for the reason that the season of seed maturity, for example, from the standpoint of the plant and the winter season of dormancy from the standpoint of environment intervene and profoundly modify culm formation.

It is a matter of value as well as of interest to compute the theoretical future rate of culm formation and see if there is any comparison within reason between the theoretical and the actual rates. Such a comparison might throw some interesting sidelights on the problem of deterioration of stand in brome-grass meadows.

EXTRAPOLATION

The method of extrapolation is simplicity itself, as one has merely to insert the proper value of x in the equations to secure the theoretical number of culms at any future period. The theoretical number of culms for a period of one year's steady growth will be found, and to do this x will have to be assigned the value of 322. This gives the number of culms at the specified time as 2,213, an increase of 2,097, or over 1,800 per cent, since the last actual count made September 11, 1916. An inspection of figure 1 indicates there would be a very rapid rate of increase after this date.

The areas of the 97 plants were computed from their diameters at the last counting date. At that time each plant occupied on an average 284.25 square cm. On the same basis the theoretical plants, after a year's steady growth, would cover an area of 5,244.241 square cm. Stated in diameters, the measurements would be 19.02 and 81.71 cm. in diameter, respectively, for the last counting date in 1916 and after the lapse of one year. The use of equation I would have increased the diameter of the theoretical plant only about 5 cm. It is worth noting that the plants discussed in this article were not far removed from the calculated size shortly after the beginning of the growing season in 1918. At that time the plants were still deficient about 1 dcm. or more in diameter from the theoretically estimated size. Assuming five months each season for rather rapid culm formation, it is evident that the given equations did not assume too much, provided culm formation proceeds the third season as rapidly as during the first and second seasons.

The foregoing does not imply, of course, that the number of culms during the third season was the number deducible from the equation. A plant may have a certain area at the close of the growing season. Such area will receive constant area increments during the next growing season. The original area also will be replenished with culms, but there is no precise knowledge at all as to the comparative number of culms for the two consecutive seasons upon the original area. Again, with certain plants, after the first season, deterioration begins within the original area, and such area of deterioration gradually enlarges. Within such an area the number of culms would be very evidently less than before deterioration began.

ANALYSIS OF CULM FORMATION

An examination of the data secured indicates that a closer analysis is possible than is given above. At the first count, as previously indicated, the plants fell into five groups in regard to number of culms. As but two plants had five culms, this group will be disregarded in the present analysis. There are thus left four groups constituted as follows:

CULMS PER PLANT.	NUMBER OF PLANTS.
1	28
2	24
3	28
4	15
Total	95

The mean was secured for each group for each counting date. The means of the four series are shown in Table II.

TABLE II.—*Means of number of culms per plant with initial culmage from 1 to 4*

Date.	Mean number of culms.			
	1	2	3	4
June 19	1. 57	2. 92	3. 61	4. 27
24	3. 46	5. 08	6. 18	5. 87
29	5. 79	8. 46	12. 11	12. 33
July 5	6. 71	9. 67	14. 46	15. 53
10	7. 75	12. 29	17. 18	18. 73
15	9. 71	15. 00	19. 96	21. 93
20	14. 04	21. 88	25. 43	28. 93
25	20. 29	29. 42	35. 79	39. 80
31	24. 39	32. 58	41. 18	46. 73
Aug. 5	31. 50	39. 63	52. 29	58. 27
10	38. 18	49. 21	65. 39	68. 00
15	48. 18	62. 83	83. 71	86. 60
22	54. 32	71. 67	95. 43	97. 47
26	63. 86	83. 63	109. 07	109. 60
31	75. 64	94. 21	122. 82	123. 93
Sept. 5	90. 04	107. 42	143. 04	140. 40
11				

A casual examination of the four series of means in Table II shows that, so far as number of culms per plant is concerned, there is a gradual increase in differences except between the two series initialing at 3 and 4. The series initialing at 4 has fewer culms per plant at the third and last count. Apparently the last series shows a lag, in comparison with what one observes among the first three series. A possible explanation of this lag will be given later, but attention is called again to the fact that the last series included only about half the number of plants found in the other series. To render the four groups more graphic they are plotted and shown in figure 2.

It seems strange at first sight that two groups of plants differing by only a single culm at an early stage of their existence should later come to show such a marked difference in this regard. It seemed possible that an explanation might be forthcoming on the basis of different degrees of heterosis; that is, the different degrees of growth vigor due to the differences in heterozygosis or amount of hybridity.

Two assumptions would be necessary, in connection with the well-known fact that brome grass is a normally inter-pollinating plant. One assumption would be that in a random sample of brome-grass seedlings some of them would possess a considerably higher degree of heterosis than others and that this difference in heterosis would result, in one direction, in a varying number of culms produced per plant. It is extremely probable that brome-grass seedlings taken at random have genotypes of sufficient diversity to be responsible for certain differences in vigor of growth which might easily find expression in a difference in number of culms. Moreover, one would look for an initial difference of this kind to be cumulative in character, as the difference in vigor would persist through the life history of the plant. The foregoing expla-

nation, while perhaps attractive, is rather too speculative for experimental proof.

The problem at hand is simply the explanation of the increasing difference in number of culms per plant for the various groups with different initial culmage, assuming, from lack of knowledge, that initial differences were purely accidental (enviromental) rather than genotypical.

If an explanation is at hand for the increasing diversity, starting from initial differences in the seedlings (environmental in character), there

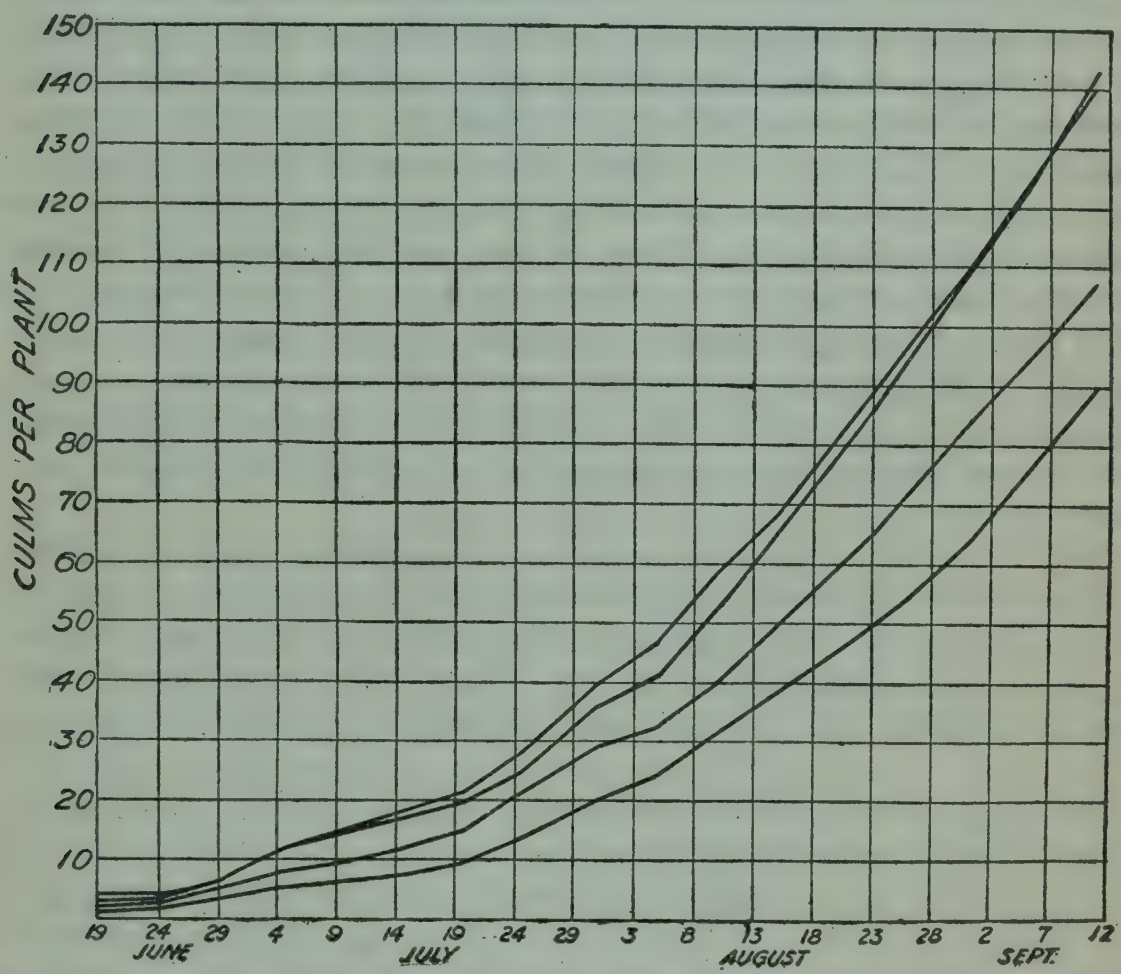


FIG. 2.—Plotting of average number of culms per plant at different dates of counting, arranged in four groups with regard to initial culmage.

would scarcely be room for additional explanations based on genotypical diversity.

In formulating another explanation, one may start with a general idea of the mechanics, let us say, of culm formation. For example, if a single culm is present, sooner or later an additional culm will be added, thus doubling the number. Later on the plant thus constituted will receive added increments, but the rate of increase of these future increments will to a considerable extent have to be assumed. However, it is certain that each culm, new and old, can not go on adding one more culm to itself indefinitely at each stage of culm accretion. Such a rate would soon be self-inhibiting from purely spatial considerations. Very

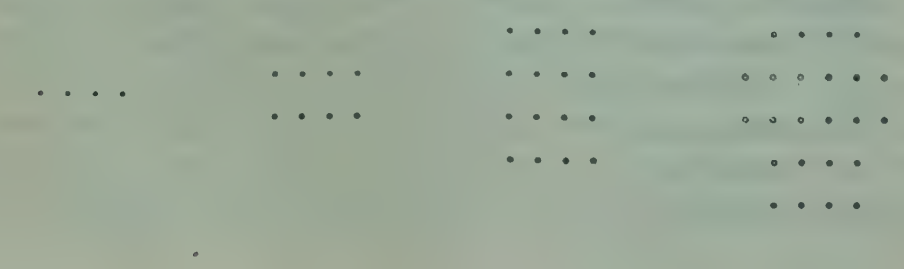
superficial observations lead to the necessary conclusion that culm formation in the interior of a grass plant, within the boundary of a more or less narrow peripheral ring, is sooner or later slowed down to a nearly zero rate. A few culms in the interior may be formed, but certainly anything like continued doubling is quite impossible. Culm increase is mainly always centrifugal in character.

USE OF CONVENTIONAL PLANTS

Relative merely to the rate of culm formation, four conventional plants were conceived with one, two, three, and four culms per plant, respectively. It was recognized that the rate of culm increase would presumably lessen as the total number of culms per plant increased. Even the minimum rate of increase of a 1-culmed plant could not be maintained for any length of time, as was pointed out above. In dealing with the four conventional plants just mentioned, notice was taken only of the number of culms added at each culm accretion period. The time element, the time elapsing between successive accretion periods, could be modified at will. With the groups of plants actually in hand, the time element was a factor to be reckoned with. As the time between counting periods was lessened the number of culms added each period was lessened, and contrariwise.

With the foregoing ideas in mind, the four conventional plants were assumed to double their entire culmage at definite intervals so long as the entire culmage was peripheral in character. In the more advanced states, when a differentiation became existent between the interior and peripheral areas, only the peripheral culms were conceived to produce sister culms. It is obvious that as a plant increases in size, the peripheral ring, assuming it to remain of constant thickness, increases in area relatively more slowly than the interior area of the plant. In terms of rate of culm formation, this would mean that with the increase in size of plant, coincident with its advancing age, the ratio of culm increase would constantly decrease as the total number of culms per plant increased.

The earlier stages of the conventional plant initialing with four culms is presented. This one suffices, since the plants initialing at one and two culms pass through the 4-culm stage. The plant initialing with three culms has its stages somewhat modified. Indicating culms by dots we have the following scheme:



From the foregoing explanation, it is seen that only the peripheral culms produce sister culms, and at any stage of increase a peripheral culm produces only one sister culm. The series resulting when the initial culmage is 4 is obviously as follows: 4, 8, 16, 28, 44, 64, etc., the first difference being continually augmented by 4. The general expression for this series is

$$y = 2x^2 - 2x + 4.$$

Now, the point in question is whether the results obtained in the field agree reasonably well with the figures obtained from the conventional plants. In the first place, one is dealing with the phenomenon of growth, which develops in a continuous manner. However, since the new growth is not recognized until it attains a certain stage of development in a culm of certain height, the accretions may be considered as being discrete in character. The method of recording the accretions, by counting, was necessarily discrete. The time elapsing between was chosen in a purely arbitrary manner, and in any explanation or symbolical description of rate of culm formation it would be purely a matter of choice if the length of time between countings were modified in one way or another. If the time periods were to be arbitrarily reduced from those used in the field, the number of culms appearing at each counting would have to be interpolated in so far as the new periods were concerned. The same would hold true if the time periods were increased unless, indeed, the new time periods were made some multiple of those actually used. As a matter of convenience, it seems better to double the period length between countings when comparing conventional plants with the actual ones. The number of countings would therefore be reduced to nine with an interval of about 10 days between successive counts.

In Table III are given the counts made upon the four series of plants based upon 10-day intervals, and for comparison there are presented the figures from the four conventional plants having a rate of culm increase according to the scheme outlined.

TABLE III.—*Comparison in rate of culm formation between four groups of plants and four conventional plants*

Date.	Number of culms.							
	A	B	A	B	A	B	A	B
June 19....	1	1	2	2	3	3	4	4
29....	3.46	2	5.08	4	6.18	6	5.87	8
July 10....	6.71	4	9.67	8	14.46	12	15.53	16
20....	9.71	8	15.00	16	19.96	22	21.93	28
31....	20.29	16	29.42	28	35.79	36	39.80	44
Aug. 10....	31.50	28	39.63	44	52.29	54	58.27	64
22....	48.14	44	62.83	64	83.71	76	86.60	88
31....	63.86	64	83.63	88	109.07	102	109.60	116
Sept. 11....	90.04	88	107.42	116	143.04	132	140.40	148

ANALYSIS OF TABLE III

It is evident from a casual inspection of Table III that there is a rather striking similarity between the two comparison columns, A and B, in the several cases.

Using either A or B as the standard, the deviations added with respect to sign give a sum equal to 5.11 culms for the entire number of counting periods. Reduced to the mean deviation for each counting period it is less than unity, an inconsequential amount. However, the size of the deviations must be considered; so that if they are added irrespective of sign and the mean deviation per group determined for each counting period, one finds the mean to be 2.92 culms. This mean deviation is about 6 per cent of the mean number of culms for each counting period. Considered from either of the foregoing standpoints, it appears that the agreement between the rate of culm formation of the real brome plants and the ideal rate of formation of the conventional plants is reasonably close. It is not to be understood that the plants in the field really follow the ideal method as outlined. This would be putting the case rather too strongly. It is known that the actual and conventional plants started with the same number of culms and that culm formation proceeds centrifugally in the actual plant as it does by hypothesis in the conventional plants. Finding the results in the two cases so closely in accord, one may say without much hesitation that given a certain number of groups of plants of *Bromus inermis* possessed of varying initial culmage, one may expect, with normal development, a continual increase in number of culms for each group but at the same time with a continual increasing diversity in number of culms between the various groups according to fixed rates.

While the correspondence of group A and group B, taking them as a whole, can be considered only general in character, it apparently is sufficiently close to make it seem unwise with our present knowledge to make other attempts to explain the increasing diversity in number of culms between the various groups with the advance of age of plants.

It should be observed that the number of plants in the group having four initial culms was somewhat lower than in the other groups. The lagging effect exhibited by this group may be attributed perhaps to the fact that the plants of the group did not comprise so representative a sample as did the plants of the other groups and that more than their due proportion lagged behind, thus diminishing the true mean. As a matter of fact there were a number of plants which behaved, after the first one or two counts, as though belonging genotypically to one of the groups having a smaller initial culmage. However, another explanation is possible. It may be that the effect of transplanting was more detrimental to the 4-culm plants, which were farther advanced, than to the plants not so far developed. Such an effect would be a retarding one and likely to be confused with constitutional difference.

COMPOUND INTEREST LAW

The compound interest law applied by Blackman [1] to plant growth deals with processes in which any rate of change varies as the quantity which is changed. This law evidently applies in the present case, as the larger the brome-grass plant the larger the peripheral ring which produces new culms. This law is expressed symbolically as

$$W_1 = W_0 e^{rt}$$

where W_1 is the final weight or measure of the plant, W_0 the initial weight or measure, r the rate of interest, t the time involved, and e the base of the natural system of logarithms. Obviously

$$r = \frac{\log W_1 - \log W_0}{t \log e}.$$

The rate of interest, r, appears to Blackman as an important physiological constant, for it represents to him the efficiency index of the plant as a producer of new material.

Similar efficiency indexes were calculated for culm formation in *Bromus inermis* for the four initial culmages for the various dates. Each index, or interest rate, indicates comparative average rate of growth per day for the period of growth to each respective date, using the proper initial culmage as the base in each of the four cases. The indexes are presented in Table IV.

TABLE IV.—*Efficiency indexes of culm formation with four-initial culmages as determined by the compound interest law*

Growth period.	One initial culm.	Two initial culms.	Three initial culms.	Four initial culms.
5	0.090	0.076	0.037	0.013
10	.124	.093	.072	.038
16	.110	.090	.087	.070
21	.091	.075	.075	.065
26	.079	.070	.067	.059
31	.073	.065	.061	.055
36	.073	.067	.059	.055
42	.072	.064	.059	.055
47	.068	.059	.056	.052
52	.066	.057	.055	.052
57	.064	.056	.054	.050
64	.061	.054	.052	.048
68	.059	.053	.051	.047
73	.057	.053	.049	.045
78	.056	.049	.048	.044
84	.054	.047	.046	.043

[1] Blackman, V. H. THE COMPOUND INTEREST LAW AND PLANT GROWTH *In* Ann. Bot., v. 33, no. 131, p.353–360. 1919.

From Table IV one notes a distinct rise in the indexes up to the second or third counting period and then a gradual but somewhat irregular falling to the last period. The indexes as a whole decrease in size as the initial culmage increases. For the group of plants initialing at 4 the efficiency index, or rate of interest, averages less and is uniformly less than for any of the other groups. During the early stages the rate of increase is considerably higher with the plants with fewer culms. An individual plant with 1 culm doubles its culmage at the first increase, but with a 4-culmed plant the culmage need be increased but by 25 per cent. It would seem to appear in this case that the plants with the larger initial culmage are less "efficient" than the smaller initialing group and that this diminished "efficiency" is automatically due to the larger size of plant. This condition is quite different in those cases cited by Blackman, where the small initial seed weights lead directly to a lessened efficiency index. It is not evident that the indexes dealing with culm formation in brome grass have any real organic significance.

SUMMARY

A study is made of rate of culm formation in *Bromus inermis*. The rate of increase is not fixed but takes place at an accelerating rate. An equation was developed which expresses the rate of increase in a symbolical manner. The resulting curve is of the parabolic type.

An analysis of the data shows that plants initialing at culmage 1, 2, 3, and 4 become, on the whole, increasingly more divergent in number of culms during the season. This increasing divergence may be explained from the initial culmage and the consequent mechanics of further culm formation. It is not evident that the compound interest law has any real organic significance when applied to culm formation in *Bromus inermis*.

RELIABILITY OF THE NAIL TEST FOR PREDICTING THE CHEMICAL COMPOSITION OF GREEN SWEET-CORN[1]

By CHARLES O. APPLEMAN

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INTRODUCTION

The chemical composition of green sweetcorn changes very greatly during the ripening period. The rate at which these changes occur depends upon the prevailing temperature. In warm weather the stage of ripening that furnishes the most desirable chemical composition of green sweetcorn, either for table use or packing into cans, passes very quickly. This rapidly changing composition of the corn also introduces the chief difficulty in conducting various lines of experimental work that require the sampling of ears as nearly as possible at the same stage of ripening. In practice, as well as in experimental work, it has been customary to resort to inspection of certain physical and structural characters of the corn as a basis for the prediction of the approximate chemical composition of the kernels. One of the tests frequently used in this connection, especially in experimental work, is the so-called nail test.

THE NAIL TEST

In the manipulation of the nail test the thumb-nail is thrust into the kernel, and if the exudate is milky the corn is said to be in the milk or best edible stage. If, on the other hand, dough is forced out of the kernel by the nail the corn is said to be in the dough stage and is considered too far advanced in ripening to give the best quality to green corn. In order to determine within what limits the chemical composition of the corn can be predicted by the nail test, it was applied at different stages of ripening, and samples were taken from the ear for analyses. In this study four stages were indicated by the nail test and designated as follows:

(1) The premilk stage. The exudate from the kernels in this stage is cloudy or opalescent, but not white and milky.

(2) The typical milk stage. The exudate is milky, and the kernels are white.

(3) The early dough stage. In this stage a little pressure by the nail is required to force out the creamy contents, which also contain a little dough.

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[1] Published with the approval of the Director of the Maryland Agricultural Experiment Station.

A large part of the experimental data in this paper was compiled from other problems, in which both John M. Arthur and S. V. Eaton, former assistants in this laboratory, contributed to the analytical work.

(4) The dough stage. At this stage of ripening only dough is force out of the kernels by the nail, and the kernels are also beginning to show considerable yellow color.

EXPERIMENTAL DATA

Stowell's Evergreen corn furnished the material for this study, which covered two crops. The first crop was planted so that it ripened in August. The average hourly mean temperature for the ripening period was 83° F. This crop will be spoken of as the early crop. The second crop ripened in the cool autumn. The average hourly mean temperature for the ripening period was 65°. Besides being cool, the weather was usually cloudy and damp. This crop of corn will be designated as the late crop.

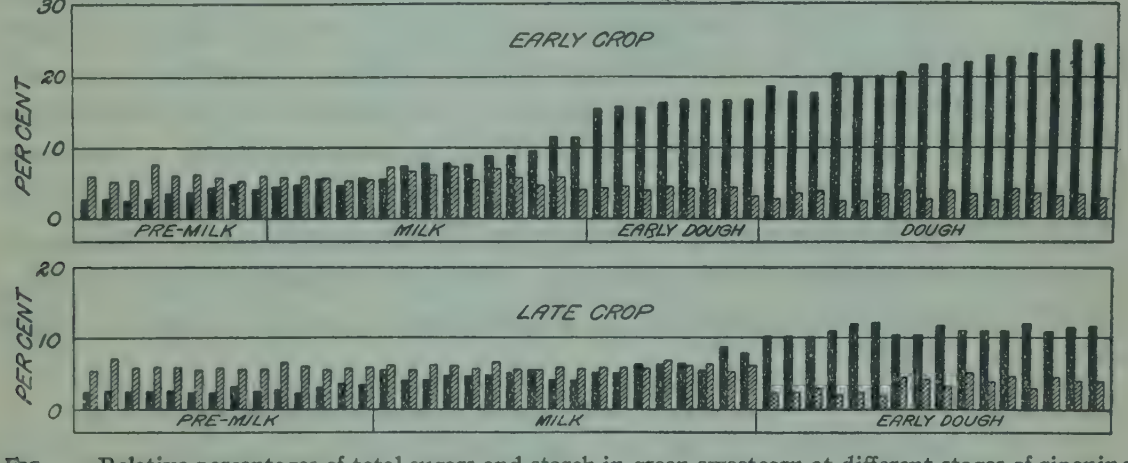

FIG. 1.—Relative percentages of total sugars and starch in green sweetcorn at different stages of ripening as indicated by the nail test. The solid columns represents the starch percentages, and the broken columns the percentages of total sugars. Each pair of columns represents a single ear. The ears for each stage of ripening are arranged according to the percentage of water in the kernels, beginning with the highest water content at the left of the figure.

The experimental results are set forth in Tables I, II, and III and in figure 1. Only moisture and carbohydrate determinations are given, as these constituents are the ones chiefly involved in the changes in percentage composition of the corn during ripening.

TABLE I.—*Percentage composition of sweetcorn at different stages of ripening as indicated by the nail test. Early crop*

Nail test.	Number of tests.		Moisture.	Total sugars.	Starch.	Ratio of sugar to starch.
Premilk	9	Maximum	87.52	8.06	4.93	1.903
		Minimum	83.01	5.15	2.33	
		Average	85.10	6.26	3.29	
Milk	18	Maximum	84.94	7.56	11.94	.750
		Minimum	73.25	4.08	4.27	
		Average	80.16	5.79	7.72	
Early dough	9	Maximum	73.50	4.61	19.57	.239
		Minimum	66.24	2.94	15.15	
		Average	71.07	3.91	16.35	
Dough	18	Maximum	69.59	4.17	25.42	.146
		Minimum	58.76	2.12	17.80	
		Average	63.92	3.17	21.62	

TABLE II.—*Percentage composition of sweetcorn at different stages of ripening as indicated by the nail test. Late crop*

Nail test.	Number of tests.		Moisture.	Total sugars.	Starch.	Ratio of sugar to starch.
Premilk	14	Maximum	90. 58	6. 78	3. 56	2. 126
		Minimum	85. 51	5. 21	2. 14	
		Average	88. 75	5. 76	2. 71	
Milk	16	Maximum	85. 40	6. 68	8. 73	1. 054
		Minimum	80. 56	5. 02	3. 89	
		Average	83. 54	5. 81	5. 51	
Early dough	16	Maximum	82. 56	4. 93	12. 66	. 300
		Minimum	74. 13	1. 58	10. 19	
		Average	77. 95	3. 38	11. 24	

TABLE III——*Average percentages of Tables I and II calculated to dry basis*

Crop.	Premilk stage.		Milk stage.		Early dough stage.		Dough stage.	
	Total sugar.	Starch.	Total sugar.	Starch.	Total sugar.	Starch.	Total sugar.	Starch
Early	42. 013	22. 080	29. 183	38. 911	13. 515	56. 519	8. 786	59. 922
Late	51. 200	24. 080	35. 297	33. 475	15. 328	50. 975	.....	.....

EARLY CROP

The percentages of moisture, total sugar, and starch were fairly uniform in the kernels from the ears of the early crop classified by the nail test as being in the premilk stage. The same was true of the ears picked in the early dough stage. The ears picked when the nail test indicated the typical milk stage showed, however, a considerable range in the percentages of the foregoing constituents, indicating extensive carbohydrate transformation in the kernels during the milk stage. The ratios of total sugar to starch ranged from 1.428 to 0.387.

During the period when the corn gave the typical dough test the percentages of starch gradually increased. The percentages of total sugar were somewhat irregular but uniformly low. The dough stage covered the period from the end of the early dough stage until the moisture in the kernels was reduced to about 60 per cent. At this point the carbohydrate transformations had practically reached equilibrium positions, and thereafter the percentages of sugars and starch increased only as water was lost by evaporation. Calculated on wet basis, the percentages of total sugar for each stage of ripening showed less variation than the percentages of starch.

LATE CROP

The percentage composition of the kernels for each stage of the late crop was much more uniform than the corresponding stage of the early crop. The percentages of starch in both the milk and early dough stages

were lower in the late crop than in the early crop. The ripening processes proceed to nearly equilibrium positions independently of the rate of water loss by evaporation. On account of the cool, damp weather, the late crop came to the early dough stage with a relatively high percentage of water, making the percentage of starch lower than at the same stage in the early crop. By comparing the percentage of total sugar and starch based on dry weight and also the ratios of total sugars to starch, it will be noted that the late crop in the milk and early dough stages was not quite as far advanced in ripening as the early crop in the same stages. A smaller ratio of sugar to starch indicates a more advanced stage of ripening.

SUMMARY AND CONCLUSIONS

Based upon the nail test the ripening period of sweetcorn was divided into four stages—the premilk, typical milk, early dough, and dough stages. The application of the nail test in classifying green corn into these four stages of ripening is described.

The reliability of the nail test as a means of predicting the chemical composition of green corn at different stages of ripening is shown by the results of chemical analyses of the corn after the nail test was applied.

The reliability of the nail test is influenced by the rate of ripening and also by the rate of water loss by evaporation.

The corresponding stages of crops ripening under different climatic conditions vary both in uniformity of composition and average percentage composition. The percentage composition of the carbohydrates in the milk stage of a late crop was much more uniform than in the same stage of an early crop.

The nail test is most reliable when applied to crops which ripen slowly in the cool autumn. These crops are most suitable for experimental work requiring the sampling of ears as nearly as possible at the same stage of ripening.

In each of the ripening stages except the dough stages the percentage of total sugar was more constant than the percentage of starch.

As ripening proceeds, the increase in the percentage of starch is much greater than can be accounted for by the decrease in the percentage of sugars. From the beginning of kernel formation until the end of the ripening period there is a continual movement of sugar from the plant into the kernels, where it is transformed into starch.

TRANSMISSION OF SOME WILT DISEASES IN SEED POTATOES[1]

By M. B. McKay

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Among the diseases of the potato plant (*Solanum tuberosum* L.), the wilt diseases are the most widely known and are reported to cause the greatest loss the whole country over. There are several fungi which cause wilt of potato plants, but the most important and most frequently mentioned are *Fusarium oxysporum* Schlecht. and *Verticillium albo-atrum* Reinke and Berthold. These parasites have been known for many years as a cause of disease in potatoes, and although many important facts concerning their nature and control have been learned by the different investigators who have studied them, there still remain points of interest and value concerning which there is a lack of adequate and definite knowledge and which will require much additional work to be completely cleared up. The investigations reported here were begun in order to secure some knowledge of the importance of these wilt diseases in Oregon and to determine the extent to which the fungi causing them are transmitted from year to year in seed potatoes. *Fusarium radicicola* Wollenw. has during this work been frequently isolated from the stem-end vascular region of potato tubers that in appearance could not be distinguished from those affected by a known wilt fungus. It has been necessary, therefore, to include this fungus in this study even though it is not known to be a cause of wilt of potato plants. Incidentally, also, some data have been collected on the natural occurrence of these organisms in the soil.

This work was begun in 1916 and continued to the end of 1920. It has been conducted mainly in the vicinity of Corvallis, though field observations have shown that the organisms studied performed in very much the same way throughout western Oregon. However, the results secured probably could not apply in their entirety to the eastern part of the State where the soil, climatic, and other conditions are very different. The work was begun with the supposition that *Fusarium oxysporum* was the organism chiefly responsible for potato-wilt in this region, but as the investigation has progressed it has been found that *Verticillium albo-atrum* is much the more serious of the two and that the former organism is relatively unimportant in western Oregon. Some of the tests begun for the purpose of determining the length of rotation necessary to avoid wilt infection from the soil can not be completed before a few more seasons of study. The work on the transmission of the organisms in seed potatoes has been terminated, however, and the results secured are given here.

[1] Published with the permission of the Director of the Oregon Agricultural Experiment Station.

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TRANSMISSION OF ORGANISMS IN SEED POTATOES

MATERIALS AND METHODS

In 1916 several lots of diseased and healthy potatoes of different varieties and from several localities were secured as follows: Seven lots of diseased potatoes—one each of American Wonder, Burbank, Early Rose, Up-to-Date, and Producer from three localities in Oregon; one lot of Rural New Yorker from Colorado; and one lot of Sir Walter Raleigh from Michigan; and six lots of healthy potatoes—one each of Burbank, Uncle Sam, and Gold Coin from Oregon, and one each of Burbank, Rural New Yorker, and Irish Cobbler from Wisconsin. These tubers were tested for the presence or absence of organisms in the stem-end vascular region in the following manner: The potatoes were weighed, numbered, washed, treated with mercuric chlorid 1 to 1,000 for two hours, rinsed through two lots of boiled and cooled water, spread out for one day in the laboratory to dry, placed in clean paper bags, and later cultured as follows: With a flamed and cooled scalpel a small cone of tissue about ½ inch in diameter and ⅛ inch deep was removed from around the point of stolon attachment. With another flamed and cooled scalpel a piece of the inner exposed vascular tissue, together with some surrounding parenchyma tissue, was taken from the discolored area (Pl. 139, A, B) when present, or from apparently normal tissue when discoloration was not present. This was placed in a test tube containing a moist sterilized sweet clover stem and incubated in the laboratory exposed to strong north light. As a rule a pure culture of whatever organism was present was secured (Pl. 141, B), though occasionally more than one organism was isolated. These organisms could often be identified directly from the original isolation culture, though always in the case of *Fusarium* spp. transfers were made to tubes of sweet clover stems, steamed potato cylinders, and steamed rice for final identification. By means of growth characters, colors, and characters of the spores which developed on these media, many of the species, including *F. oxysporum*, *F. radicicola*, *F. solani* (Mart.) Sacc., *F. discolor* App. and Wollenw., *F. discolor* var. *sulphureum* (Schlecht.) App. and Wollenw., *F. coeruleum* (Lib.) Sacc., and *F. culmorum* (W. G. Sm.) Sacc. could be determined by the methods of Wollenweber (13)[1] and Carpenter (2). Occasionally other species of Fusarium were encountered with which the author was not familiar and which were not determined.

After taking cultures the tubers were then prepared for planting. All tubers weighing 3 ounces or less were planted whole; those weighing from 4 to 7 ounces, inclusive, were divided into two lots, one lot being cut transversely into stem and eye halves and the other longitudinally into right and left halves; and those weighing 8 ounces and over were cut longitudinally and transversely into quarters (Pl. 139, C). All

[1] Reference is made by number (italic) to "Literature cited," p. 847-848.

the seed pieces were then planted on recently cleared land which had never grown a crop of potatoes and were given good care throughout the growing season. During the season notes on vine growth were made at occasional intervals. In the fall all the hills were dug and weighed separately. All hills grown from seed pieces from which known or suspected parasitic organisms had been isolated and a representative number of hills grown from fungus-free seed pieces as indicated by the cultures were kept for culturing in the same way that the seed tubers had been cultured in the spring. Some of this material was then kept for use as seed stock the following season, when a much larger planting on well-rotated land was made. The results obtained during the different seasons did not vary greatly but appeared to be in close accord.

RELATION BETWEEN TUBER SYMPTOMS AND OCCURRENCE OF ORGANISMS THEREIN

The presence or absence of discoloration in the vascular region of the stem end of potato tubers has rather generally been taken (*5*, *7*) as a reliable guide to the presence or absence of wilt-producing fungi, though for *Verticillium albo-atrum*, Pethybridge (*8*) has pointed out that this is far from being an infallible sign. Based on a study made in 1915 and with which the writer was associated, Edson (*4*) states that—

> In the materials studied, vascular discoloration of the stem-end tissues of Irish potato tubers was not found to be proof of the presence of parasitic fungi. Discolored bundles were often sterile, and fungi were frequently isolated from tissues which appeared normal.

The extent to which the presence or absence of discoloration can be depended on as a guide to the presence or absence of wilt-producing fungi is of importance, for it is frequently desirable to estimate the amount of wilt infection in seed potatoes merely from the appearance of the tubers. Consequently, a further study has been made of this relation in several different lots of potato tubers, of which the following may be mentioned in detail:

Six hundred Up-to-Date tubers grown in 1917 in hills attacked by *Verticillium albo-atrum* were cultured by the method outlined above. Nearly three-fourths of them (439 tubers, or 73.2 per cent) yielded *V. albo-atrum* in culture. Of these, 70.8 per cent were "browned" in the vascular region at the stem end, 19.6 per cent were "yellowed," and 9.6 per cent were "not discolored" at all. Of the 95 tubers in this lot which gave no organism in culture, 18.9 per cent were "browned" in the vascular region, 17.9 per cent were "yellowed," and 63.2 per cent were "not discolored." There were 111 tubers altogether which had no discoloration in the vascular region. Of these 54 per cent gave no organism in the cultures; 8.1 per cent gave "miscellaneous fungi" to which no importance can be attached, for they probably cause no disease of potatoes; while more than a third, 37.9 per cent, gave *V. albo-atrum*.

Of all the tubers which were "browned," 14.2 per cent gave no organism of importance, and 84.3 per cent gave *V. albo-atrum*. Thus, if this lot of tubers had been sorted for seed on the basis of the presence or absence of discoloration in the stem-end vascular region, 38 per cent of those passed as fit for seed would have been infected with *V. albo-atrum*, while 14 per cent of those eliminated for seed purposes would apparently have been entirely free from a wilt-producing organism.

In another lot of 647 tubers of seven varieties grown in 1917 from disease-free seed potatoes on soil badly infested with *Verticillium albo-atrum*, nearly half of them, or 293 tubers, were "browned" in the vascular region. These when cultured gave 55.3 per cent *V. albo-atrum*, 21.5 per cent *Fusarium radicicola*, 14.7 per cent "miscellaneous fungi," and 7.2 per

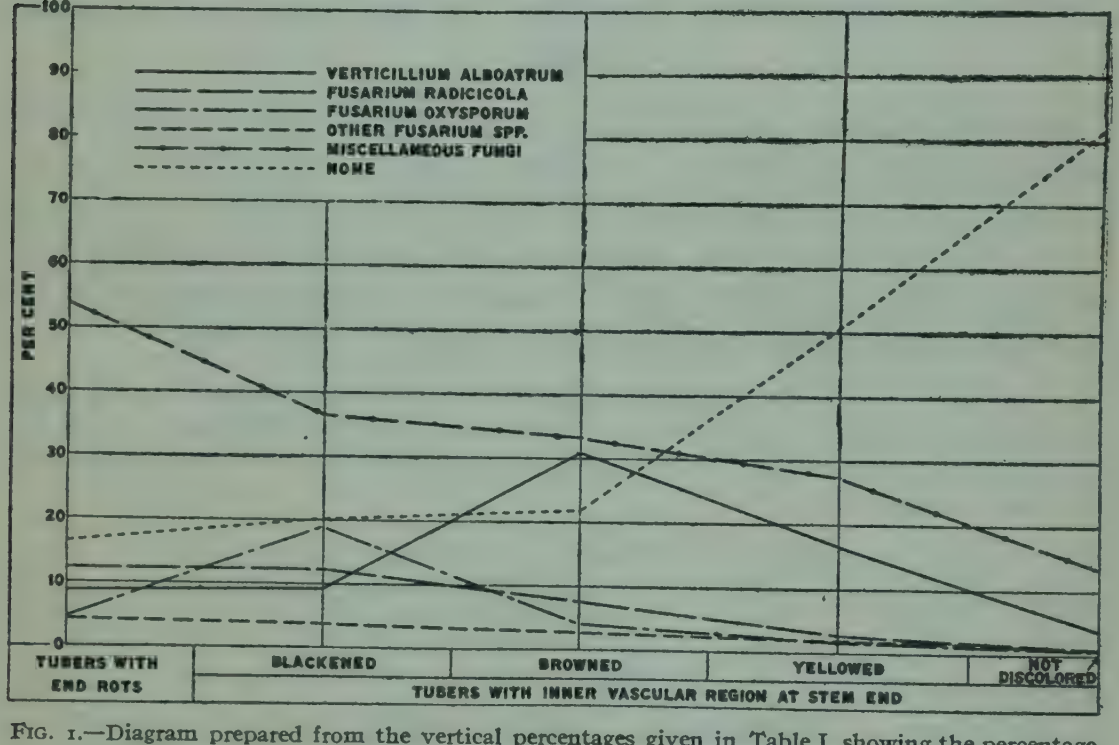

FIG. 1.—Diagram prepared from the vertical percentages given in Table I, showing the percentage occurrence of different organisms in tubers of a given discoloration.

cent no organism. Of the 260 tubers of the entire lot which gave *V. albo-atrum* in cultures, 2.7 per cent were affected by "jelly end" or slight stem-end rots, 1.9 per cent were "blackened" in the stem end, 62.3 per cent were "browned," 30.4 per cent were "yellowed," and 2.7 per cent showed "no discoloration" in the vascular region.

In another lot of 498 potato tubers of seven varieties grown in 1917, from disease-free seed potatoes inoculated at planting time with pure cultures of *Verticillium albo-atrum*, 232 tubers were "browned" in the vascular region. These when cultured gave 60.5 per cent *V. albo-atrum*, 9.6 per cent *Fusarium radicicola*, 26.5 per cent "miscellaneous fungi," and 2.1 per cent no organism. Of the 244 tubers which gave *V. albo-atrum* in cultures, 0.8 per cent were affected by "jelly end" or slight dry stem-end rots, 2.9 per cent were "blackened" in the vascular region,

82.4 per cent were "browned," 13.5 per cent were "yellowed," and only 0.4 per cent were "not discolored."

In still another lot of 258 Up-to-Date potato tubers grown by plants wilted naturally in the field with *Verticillium albo-atrum* during the season of 1916, 75 were "browned" in the vascular region. These when cultured gave 57.3 per cent *V. albo-atrum*, 9.3 per cent *Fusarium* spp., 28 per cent "miscellaneous fungi," and 5.3 per cent no organism. Of the 97 tubers which gave *V. albo-atrum* in cultures, 44.3 per cent were "browned" in the vascular region, 41.2 per cent were "yellowed," and 14.4 per cent were "not discolored."

In Table I is given a summary of practically all of the data collected during the course of this investigation on the relation of discoloration

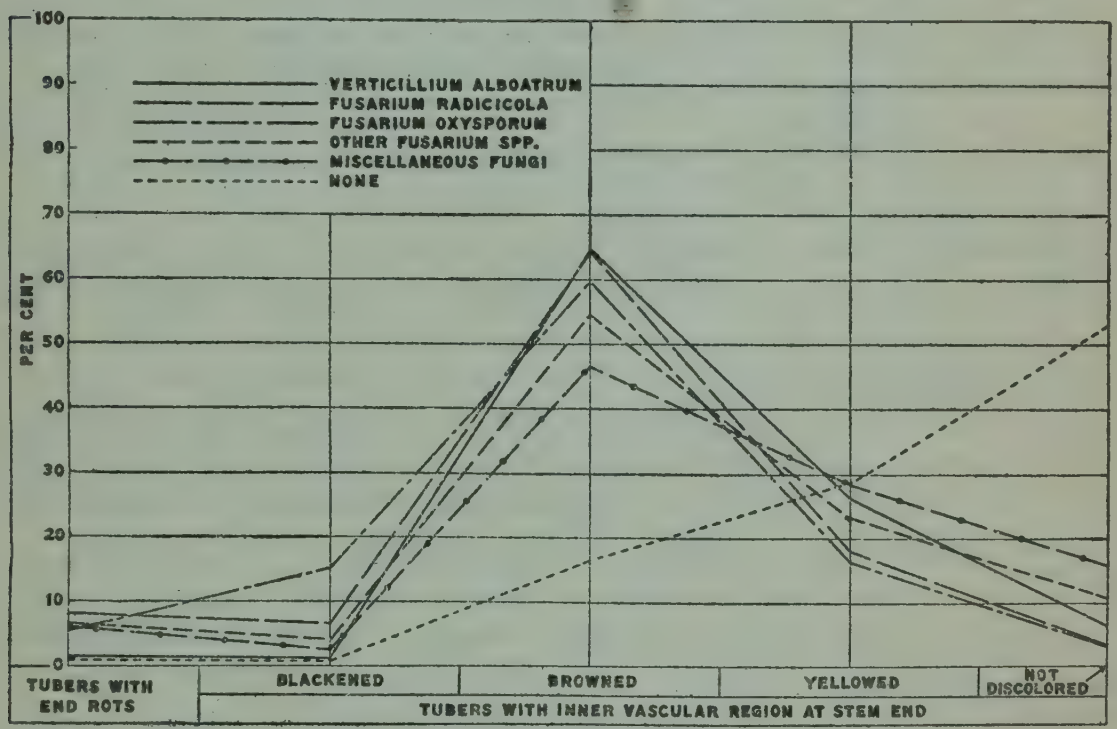

FIG. 2.—Diagram prepared from the horizontal percentages given in Table I, showing the percentage occurrence of a given organism in tubers of different discolorations.

of the vascular region to the occurrence of organisms in potato tubers. A part of the data in the table is shown graphically in figures 1 and 2. The results shown here are from a total of 12,136 tubers grown under a variety of conditions during three seasons from nine varieties and should therefore not be unusual but should serve as a basis for seemingly reliable conclusions. The table shows that *Verticillium albo-atrum* was isolated from 2.097 tubers, of which only a small percentage, 1.5, were affected by "jelly end" and slight dry stem-end rots. This is considered to be an accidental and mixed rather than a specific infection, because the great majoirty of the tubers affected by such rots gave other organisms in culture and all available data point to the fact that *V. albo-atrum* does not cause a specific rot of potato tubers. Also, only a small percentage, 1.1, were "blackened" in the vascular region. The

great majority of the tubers, 64.6 per cent, were "browned"; a large portion, 26.2 per cent, were "yellow" while a comparatively small though appreciable number, 6.6 per cent, were "not discolored." Thus the data indicate that this fungus when present in potato tubers is accompanied in the majority of cases by distinct discoloration but that frequently the discoloration is very light or even entirely absent and that therefore discoloration of the tubers is not a reliable basis for judging whether or not this fungus is present.

TABLE I.—*Relation between stem-end vascular-tissue discoloration and occurrence of organisms in potato tubers grown during the seasons of 1915, 1916, and 1917, under various conditions, some by diseased and others by healthy plants*[a]

Organisms isolated.	Tubers with—					Totals.
	"Jelly-end" and slight dry stem-end rots.	Inner vascular region at stem end—				
		Blackened.	Browned.	Yellowed.	Not discolored.	
Verticillium albo-atrum.	8.8 31 1.5	9.1 22 1.1	30.7 1,355 64.6	16.5 550 26.2	3.6 139 6.6	17.3 2,097 100
Fusarium radici-cola.	12.2 43 8.3	12.1 29 5.6	7.5 331 64.2	2.8 94 18.2	0.5 19 3.7	4.2 516 100
Fusarium oxy-sporum.	4.5 16 5.4	18.8 45 15.2	4.0 176 59.7	1.4 48 16.3	0.3 10 3.4	2.4 295 100
Other Fusarium species.	4.2 15 6.9	3.7 9 4.1	2.7 119 54.3	1.6 52 23.7	0.7 24 11.0	1.8 219 100
Miscellaneous fungi.	53.9 191 6.1	36.3 87 2.8	33.2 1,463 46.4	27.2 906 28.7	13.3 506 16.0	26.0 3,153 100
None.....	16.4 58 1.0	20.0 48 .8	21.9 969 16.5	50.5 1,680 28.7	81.6 3,101 53.0	48.3 5,856 100
Totals.....	100 354 2.9	100 240 2.0	100 4,413 36.4	100 3,330 27.4	100 3,799 31.3	100 12,136 100

[a] In each box the middle set of figures shows the number of tubers which in culture gave the results indicated in the headings of the intersecting columns; the lower right set of figures gives the percentage this number is of the total tubers represented in the horizontal column; and the upper left set gives the percentage of the total tubers in the vertical column.

Fusarium radicicola was isolated from 4.2 per cent of all the tubers cultured and included in the table. It occurred to a greater extent than *Verticillium albo-atrum* in the "endrot" and "blackened" tubers, the percentages for *F. radicicola* being 8.3 and 5.6, and for *V. albo-atrum* 1.5 and 1.1, respectively; and much less in the "yellowed" and "not discolored" tubers, the percentages being 18.2 and 3.7 for the former fungus, and 26.2 and 6.6 for the latter, respectively. The majority, 64.2 per cent, of the tubers which gave this fungus were "browned" in the vascular region. From these figures it must be concluded that this fungus occurs rather frequently in potato tubers and that it causes a somewhat heavier discoloration than *V. albo-atrum* in the invaded tissues. This latter fact is well shown in figure 2, in which at the left of the center, where the results from the heavily discolored tubers appear, *F. radicicola* occupies a higher position than *V. albo-atrum*, and at the right of the center, where the results from the lightly discolored tubers are given, it occupies a lower position than *V. albo-atrum*.

Fusarium oxysporum has not been isolated during the course of this investigation as frequently as either of the other two organisms already mentioned, having been secured from 295 tubers, or only 2.4 per cent of all the tubers cultured. Fifteen and two-tenths per cent of the tubers from which this fungus was isolated were "blackened" and only 3.4 per cent were "not discolored," as compared to 5.6 per cent and 3.7 per cent, respectively, for *F. radicicola* and 1.1 per cent and 6.6 per cent, respectively, for *Verticillium albo-atrum*. A smaller percentage of the tubers from which *F. oxysporum* was isolated were "browned" than was true of those from which *F. radicicola* and *V. albo-atrum* were secured, the percentages being 59.7 as compared to 64.2 and 64.6, respectively. Thus it appears that *F. oxysporum* caused even heavier discoloration in the tubers than either of the other two organisms. Although this is contrary to statements that are often made in literature (*5*, *7*), the results secured during the course of this investigation have been consistent on this point throughout, and it is believed that they are quite representative of the facts.

Several determined and undetermined species are grouped in Table I under "other Fusarium species," some of which may be mentioned on account of their economic importance. *Fusarium trichothecioides* Wollenw. was isolated in April, 1916, from one tuber which was "browned" in the stem-end vascular region. There was no rot present in this tuber, although other tubers in the same lot were affected by "powdery dryrot" and presented the typical appearance of this disease. One stem-end seed piece from this "browned" tuber produced a normal plant, but the organism appeared in two tubers out of nine produced in the hill, one of which was affected the following spring by typical powdery dryrot, while the other was "blackened" in the stem-end vascular region though not rotted. The discoloration present in this "blackened" and the

former "browned" tuber could not be distinguished in any way from that usually present in tubers affected by *Verticillium albo-atrum* or *F. oxysporum*. *F. discolor* was isolated once from a tuber that was "blackened," eight times from "browned" tubers, and twice from "yellowed" tubers; *F. discolor* var. *sulphureum* once from a "browned" and once from a "yellowed" tuber; *F. culmorum*, twice from "browned" tubers, and *F. solani* once from a "browned" and twice from "not discolored" tubers. A rather large number, 11 per cent of these "other Fusarium species" caused no discoloration in the affected tubers, though the largest portion of them, 54.3 per cent, were present in "browned" tubers.

Under "miscellaneous fungi" are grouped all other organisms secured in the culture series. These are, so far as known, nonparasitic, with a few exceptions. *Colletotrichum solanicolum* O'Gara was isolated from 1 "blackened," 12 "browned," 2 "yellowed," and 2 "not discolored" tubers. Rhizoctonia (*Corticium vagum* B. and C.) was isolated from 6 tubers having stem-end rot, 3 of which were dry and 3 moist and rather soft, and from 8 "browned" and 1 "yellowed" tubers. *Spondylocladium atrovirens* Harz was isolated from 1 "browned," 1 "yellowed," and 2 "not discolored" tubers. The miscellaneous fungi isolated from potato tubers varied considerably from year to year, sometimes one and sometimes another predominating. So many cultures of *Naemosphaera* sp. were secured from the tubers grown one year, usually from distinctly "browned" tubers, that this organism was suspected of being parasitic. Inoculation tests, however, gave negative results. The longer the potatoes were kept in storage the larger was the number of saprophytic organisms which they gave in cultures. Some of the additional miscellaneous organisms isolated may be indicated by the generic names only—*Acremonium*, sp., *Alternaria*, sp., *Botrytis* sp., *Cephalothecium* sp., *Cladosporium* sp., *Chaetomium* spp. (2), *Helminthosporium* sp., *Mucor* sp., *Penicillium* spp. (2), *Periola* sp., *Pestalozzia* sp., *Phoma* sp., *Ramularia* sp., *Sclerotinia* sp., *Sporotrichum* sp., *Stemphyllium* sp., *Stysanus stemonitis* (Pers.) Corda, *Vermicularia* sp., *Verticillium cinnabarinum*, Reinke and Berth, and *Zygodesmus* sp. While some of these organisms may have entered sometimes as contaminations in the cultures, most of them had penetrated at least slightly into the tissues of the stem end of the tubers, usually causing some discoloration there. Seventy-five per cent of these tubers were "browned" or "yellowed," and about 9 per cent were "blackened" or had "endrot," and only 16 per cent were "not discolored."

The percentage distribution with respect to the recorded stem-end discoloration of the tubers from which "no organisms" were secured is in considerable contrast to the low percentages of "no discoloration" and the high percentages of distinct coloration in the tubers from which the different classes of organisms, as detailed above, were isolated.

The former group of tubers is the only one of the six in which a majority, 53 per cent, of the tubers showed "no discoloration." The nearest approach to this is in the group which gave "miscellaneous fungi," of which, however, only 16 per cent had "no discoloration." A little over one-fourth, 28.7 per cent, of the tubers which gave "no organism" were lightly discolored, "yellowed," and about one-sixth, 16.5 per cent, were "browned." A very small percentage were affected by "end rot" or were "blackened," 1 and 0.8 per cent, respectively.

To sum up a portion of the data in Table I, it seems clearly indicated that the presence of a discoloration in the stem-end vascular region of a potato tuber is usually though not always evidence of the presence of an organism there. Even distinct discoloration, though, does not always mean the presence of an objectionable organism, for only about 45 per cent of the tubers distinctly discolored contained wilt or other disease-producing organisms, while the other 55 per cent yielded either miscellaneous fungi of no apparent consequence or no organisms whatever. In this connection it is to be borne in mind that the cultures made are of course not a perfect index to the organisms present, as is mentioned more in detail in a later paragraph. Neither does the absence of discoloration always indicate the absence of a serious organism, for about 5 per cent of all the tubers which were not discolored gave disease-producing organisms when cultured. A general conclusion that seems to be well justified is that the variation in the degree of discoloration caused by any one fungus in different tubers is so large and the differences in the type of discoloration caused by the various fungi are so small that the character of the discolorations can not be taken as a guide to what fungus is present or even as proof that any fungus is present.

OCCURRENCE OF MORE THAN ONE ORGANISM IN THE SAME POTATO TUBER

In any consideration of the occurrence of fungous organisms in the stem end of potato tubers the question naturally arises as to whether commonly more than one organism occurs at one time in the same tuber. In order to throw some light on this point a special set of cultures was made. One lot of 150 Up-to-Date tubers grown in 1917 in hills from plants badly wilted by *Verticillium albo-atrum* were cultured from all four quarters by the sweet clover stem method already described. In this case, after the tubers were treated and dried, the outer tissues at the stem end were cut away and the end was quartered by two longitudinal cuts at right angles to each other. A portion of the end of each of these quarters, including discolored vascular tissues whenever present, was then taken and placed in tubes of culture medium for incubation. The results, given in Table II, show that 64 tubers, or 42.6 per cent, gave *V. albo-atrum* in all four quarters, one tuber gave *Penicillium* sp. in the four quarters included in this case under "miscellaneous fungi,"

and four tubers gave no organisms in any of the four quarters. This makes 69 tubers, or 46 per cent of the lot, which gave identical results from the four quarters of each tuber. Forty-six others, or about 31 per cent, gave only one organism from a part of the quarters, varying from one to three, but not from all of the four quarters, the other quarter or quarters yielding no organism. There is thus a total of 77 per cent of the tubers which did not give more than one organism from each tuber. Thirty-four tubers, or about 23 per cent, gave two separate organisms, and one tuber gave three separate organisms from different quarters of the same tuber.

TABLE II.—*Organisms isolated from the stem end of the four longitudinal quarters of 150 Up-to-Date potato tubers harvested in 1917 from hills badly wilted with Verticillium albo-atrum*

Number of quarters yielding organisms.	Number of tubers with the four quarters of the inner vascular region at the stem end discolored as follows:													Total.
	4 browned.	3 browned, 1 yellowed.	3 browned, 1 not discolored.	2 browned, 2 yellowed.	2 browned, 1 yellowed, 1 not discolored.	2 browned, 2 not discolored.	1 browned, 3 yellowed.	1 browned, 2 yellowed, 1 not discolored.	1 browned, 1 yellowed, 2 not discolored.	4 yellowed.	3 yellowed, 1 not discolored.	2 yellowed, 2 not discolored.	1 yellowed, 3 not discolored.	4 not discolored.	
4 *V. albo-atrum*	34	5	6	2	6	2	1	1	1	5	1	....	....	....	64
3 *V. albo-atrum*, 1 miscellaneous fungi	5	3	1	....	....	....	1	1	....	2	....	....	....	....	13
3 *V. albo-atrum*, 1 none	7	2	12	3	2	....	....	1	....	2	....	....	....	....	29
2 *V. albo-atrum*, 1 *F. oxysporum*, 1 miscellaneous fungi	1	....	....	....	....	....	....	....	....	....	....	....	....	....	1
2 *V. albo-atrum*, 1 *F. oxysporum*, 1 none	....	1	....	....	....	....	....	....	....	....	....	....	....	....	1
2 *V. albo-atrum*, 2 miscellaneous fungi	3	....	....	1	....	....	....	....	....	....	....	....	....	....	4
2 *V. albo-atrum*, 1 miscellaneous fungi, 1 none	....	....	2	....	....	....	1	1	....	1	1	....	1	3	10
2 *V. albo-atrum*, 2 none	1	....	1	....	....	1	....	....	....	....	2	....	....	1	6
1 *V. albo-atrum*, 3 *F. oxysporum*	1	....	....	....	....	....	....	....	....	....	....	....	....	....	1
1 *V. albo-atrum*, 2 *F. oxysporum*, 1 none	....	1	....	....	....	....	....	....	....	....	....	....	....	....	1
1 *V. albo-atrum*, 1 *F. oxysporum*, 2 none	....	....	....	....	....	....	....	....	....	....	....	....	1	....	1
1 *V. albo-atrum*, 1 *F. radicicola*, 2 none	....	....	....	....	....	....	....	....	....	....	....	....	....	1	1
1 *V. albo-atrum*, 3 miscellaneous fungi	....	1	....	....	....	....	....	....	....	....	....	....	....	....	1
1 *V. albo-atrum*, 2 miscellaneous fungi, 1 none	....	....	....	....	....	....	....	....	....	....	1	....	....	....	1
1 *V. albo-atrum*, 3 none	....	....	1	1	....	....	....	....	....	....	....	1	1	....	4
4 miscellaneous fungi	1	....	....	....	....	....	....	....	....	....	....	....	....	....	1
3 miscellaneous fungi, 1 none	1	....	....	....	....	....	1	....	....	....	....	....	....	....	2
2 miscellaneous fungi, 2 none	....	....	1	....	....	....	....	....	....	1	....	....	....	1	3
1 miscellaneous fungi, 3 none	....	....	....	....	....	....	....	....	....	....	....	....	....	2	2
4 none	....	....	....	....	....	....	....	....	....	....	....	....	....	4	4
Totals	54	13	24	7	8	3	4	4	1	11	5	1	3	12	150

Table II shows five tubers from which both *Verticillium albo-atrum* and *Fusarium oxysporum* were isolated in cultures from different quarters. The cultures from one of these tubers are shown in Plate 141, B. This offers a possible explanation of some otherwise confusing results secured during the course of this investigation. For instance, in a few cases *F. oxysporum* was isolated from seed potatoes which when planted in clean soil gave rise to plants that wilted from attacks of *V. albo-atrum*. In cases of this kind the latter fungus may also have been present in

the seed potato when planted and, being the more vigorous organism of the two in this locality, caused the death of the plant. The indication at least is that while the great majority of tubers give only one organism in culture there are not uncommonly also a number which give two or occasionally more organisms, even two wilt-producing organisms, from different portions.

In considering the data that have been presented in Tables I and II, the question arises as to the accuracy of the information derived from the isolation cultures where only one culture is made from each tuber. It is quite likely that a number of the tubers recorded in Table I from which no organism was secured had, as a matter of fact, been invaded by some organism. This would seem to be particularly true of those affected by endrot. Yet in general the results seem to be in accord with probability. For instance, a very much larger percentage of all of the "not discolored" tubers than of those that were "yellowed" or "browned" gave no organism, the percentages being 81.6, 50.5, and 21.9, respectively. It has already been noted that of the lot of tubers referred to in Table II from each of which four cultures were made, 69 tubers, or 46 per cent, gave identical results from the four quarters of each tuber. Forty-eight others, or about 32 per cent, gave identical organisms in three of the quarters of each tuber; and 26 tubers, or about 17 per cent, in two of the quarters of each tuber. From the standpoint of chance, one single culture made from each of these 143 tubers would have given an accurate index to the organisms present in a total of 118 tubers, or 82.5 per cent of the group. A single culture made from each of the remainder would not necessarily have represented the exact conditions, although even here it would have shown the real situation more often than not, for in many cases the fungi secured came from the quarter or quarters having the most discoloration, and where only one culture was made it was always made from the most heavily discolored portion. From these data it is believed that the method for making the cultures in this investigation, though offering some chance of failure in securing correct evidence as to the organisms present, is still at least reliable enough to give a very good indication of the facts. It has seemed also to be the most practical method available where a large number of cultures had of necessity to be made in a comparatively short time and where it was desired, as in this case, to keep the tubers in as nearly natural condition as possible for purposes of planting for further records.

RELATION BETWEEN SIZE OF POTATO TUBERS AND OCCURRENCE OF ORGANISMS THEREIN

The question arises as to whether tubers of a certain general size are more likely to be free from internal infection than others and might, therefore, be selected out for planting with the expectation of getting a smaller amount of disease from them than from tubers of other sizes. There is

no theoretical reason for supposing that there would be any correlation between size and percentage of infection except that tubers from diseased plants are likely to be smaller than those from healthy plants, although late-formed tubers which would tend to be small in size would also be likely to show less infection than older ones because exposed to danger of invasion by the fungus for a briefer period. This would perhaps be counterbalanced by the presence of a larger amount of infective material in a field at the close of the season than at the start, due to the general progress of the disease.

To secure evidence bearing on this subject a tabulation was made of the data in hand, which includes results from 12,136 tubers. This is given in Table III and is partly shown in figures 3 and 4. These are the same tubers from which Table I was made, and they were grown under a variety of conditions, during three seasons from nine varieties, some by diseased

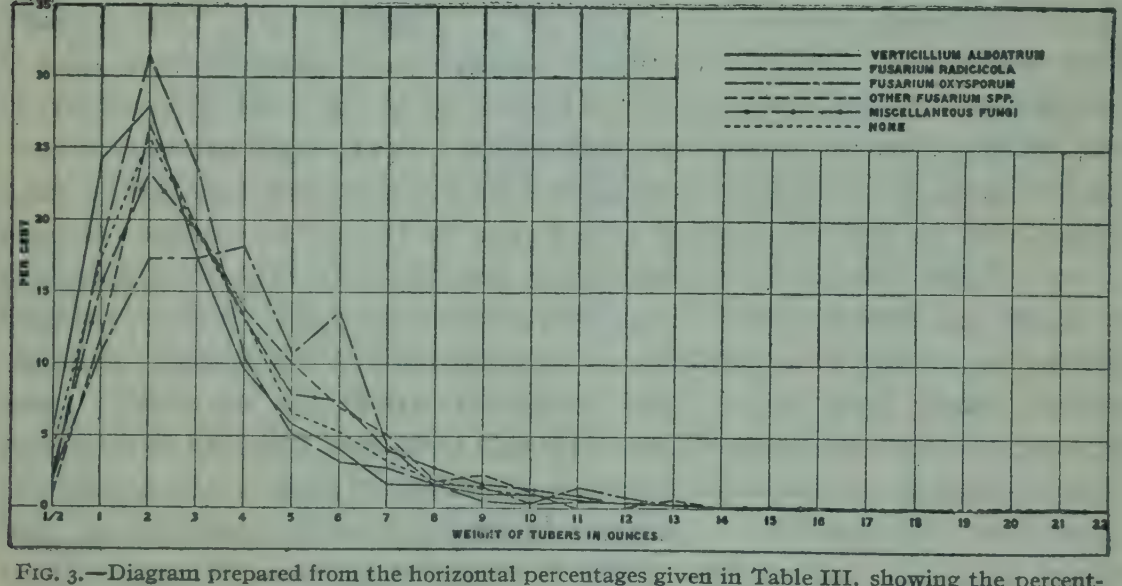

FIG. 3.—Diagram prepared from the horizontal percentages given in Table III, showing the percentage occurrence of a given organism in tubers of different weights.

and others by healthy plants. The results show that in general the organisms dealt with do not seem to occur considerably more in one size of potato tuber as measured by weight than in another, though there are a few noticeable differences. For instance, in figure 4, which shows the percentage occurrence of different organisms in tubers of a given weight, it will be seen that *Verticillium albo-atrum* was present to a smaller extent in the medium-sized tubers than in those of small or of large size, there being a steady decrease in percentage of this organism from 25.5 in the tubers weighing ½ ounce down to 9 in those weighing 7 ounces, then an increase up to 16.4 per cent in the 11-ounce tubers. And when comparisons are made between organisms from the standpoint of percentage of a given organism in tubers of different weights (fig. 3) then it will be seen that a greater proportion of all the tubers giving *V. albo-atrum* in

culture were small than was true in the case of any other organism. This relation may be further indicated by a specific comparison with the tubers which gave no organism when cultured. In the tubers weighing less than 3 ounces the percentage of all the tubers giving *V. albo-atrum* is higher than that of those giving no organism, while at 3 ounces and above the relative position of the two lots is reversed.

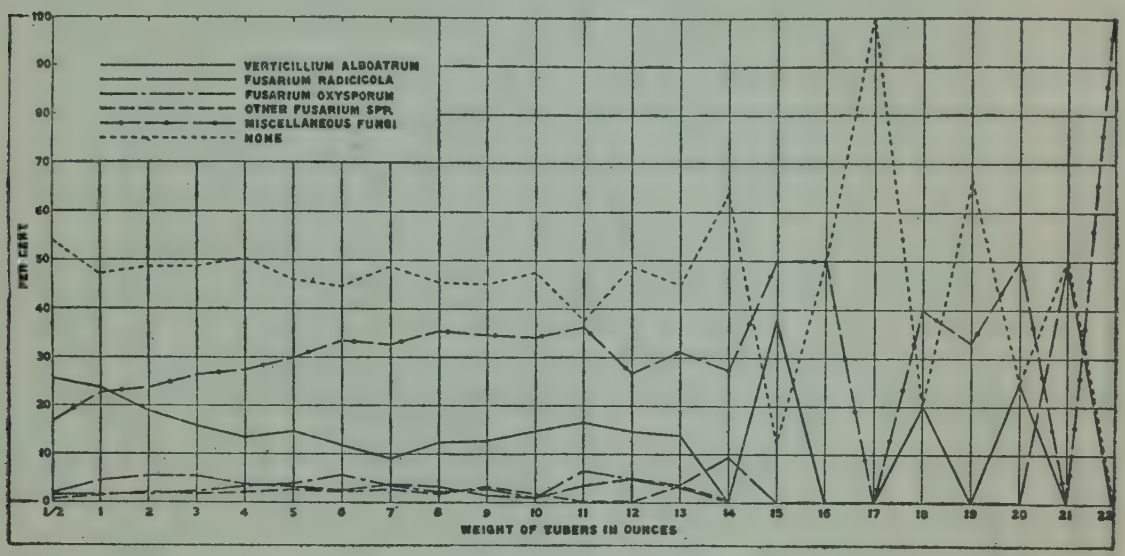

FIG. 4.—Diagram prepared from the vertical percentages given in Table III, showing the percentage occurrence of different organisms in tubers of a given weight.

Fusarium radicicola (fig. 3) occurred relatively more in the 2-, 3-, and 4-ounce tubers and less in the smaller and the larger tubers than did *Verticillium albo-atrum*. *F. oxysporum* occurred to a less extent than the other organisms in the tubers weighing 3 ounces and under and more than the other organisms in those weighing from 4 to 7 ounces. There was a gradual increase in percentage of "miscellaneous fungi" (fig. 4) from 16.6 in the ½-ounce up to 36.1 in the 11-ounce tubers. In the same range of tuber weights there was a fairly uniform decrease in percentage of "no organisms" isolated from 54.1 down to 37.7. Above 13 ounces there were so few tubers available for tabulation that the results for all the organisms isolated are variable.

The two chief lessons to be gained from these data are (1) that the smaller potatoes should not be used for seed purposes, because they are apt to contain a higher percentage of wilt infection than the medium-sized tubers unless it is known that the plants by which they were grown were free from wilt, in which case, of course, the small potatoes could be used with safety, and (2) that wilt can not be avoided merely by the selection from the bin of only the medium-sized or even the larger healthy-looking potatoes, as even these may contain a high percentage of wilt infection.

TABLE III.—*Relation between size of potato tubers, as measured by weight, and occurrence of organisms therein in tubers grown during the seasons of 1915, 1916, and 1917, under various conditions, some by diseased and others by healthy plants* [a]

Organisms isolated.	Tubers weighing (in ounces)—											
	½	1	2	3	4	5	6	7	8	9	10	11
Verticillium albo-atrum	25.5 108 5.1	23.4 506 24.1	18.9 585 27.9	15.8 366 17.5	13.2 201 9.6	14.6 122 5.8	11.9 83 4.0	9.0 35 1.6	12.4 31 1.5	12.7 19 0.9	14.7 16 0.8	16.4 10 0.5
Fusarium radicicola	1.9 8 1.6	4.3 93 18.0	5.2 162 31.4	5.3 123 23.8	3.6 55 10.6	3.1 26 5.0	2.3 16 3.1	3.6 14 2.7	3.2 8 1.6	1.3 2 .4	.9 1 .2	3.3 2 .4
Fusarium oxysporum	1.4 6 2.0	1.5 32 10.8	1.6 51 17.3	2.2 51 17.3	3.5 53 18.0	3.8 32 10.8	5.7 40 13.6	3.4 13 4.4	2.0 5 1.7	2.7 4 1.4	.9 1 .3	6.5 4 1.4
Other Fusarium species	.5 2 .9	1.2 26 11.9	1.9 58 26.5	1.8 43 19.6	2.0 30 13.7	2.6 22 10.0	2.2 15 6.9	2.8 11 5.0	1.6 4 1.8	3.3 5 2.3	1.8 2 .9	
Miscellaneous fungi	16.6 70 2.2	22.5 485 15.4	23.6 730 23.2	26.1 605 19.2	27.3 417 13.2	29.9 250 7.9	33.3 233 7.4	32.5 126 4.0	35.2 88 2.8	34.7 52 1.6	34.0 37 1.2	36.1 22 .7
None	54.1 229 3.9	47.1 1,019 17.4	48.8 1,511 25.8	48.8 1,132 19.3	50.4 769 13.1	46.0 384 6.6	44.6 312 5.3	48.7 189 3.2	45.6 114 2.0	45.3 68 1.2	47.7 52 .9	37.7 23 .4
Totals	100 423 3.5	100 2,161 17.8	100 3,097 25.5	100 2,320 19.1	100 1,525 12.6	100 836 6.9	100 699 5.8	100 388 3.2	100 250 2.1	100 150 1.2	100 109 .9	100 61 .5

Organisms isolated.	Tubers weighing (in ounces)—											Total.
	12	13	14	15	16	17	18	19	20	21	22	
Verticillium albo-atrum	14.6 6 0.3	13.8 4 0.2		37.5 3 0.1			20.0 1 0.05		25.0 1 0.05			17.3 2,097 100
Fusarium radicicola	4.9 2 .4	3.4 1 .2	9.1 1 .2				20.0 1 .2			50.0 1 .2		4.2 516 100
Fusarium oxysporum	4.9 2 .7	3.4 1 .3										2.4 295 100
Other Fusarium species		3.4 1 .5										1.8 219 100
Miscellaneous fungi	26.8 11 .3	31.1 9 .3	27.3 3 .1	50.0 4 .1	50.0 5 .2		40.0 2 .1	33.3 1 .03	50.0 2 .1		100 1 .03	26.0 3,153 100
None	48.8 20 .3	44.9 13 .2	63.6 7 .1	12.5 1 .01	50.0 5 .1	100 3 .1	20.0 1 .01	66.7 2 .03	25.0 1 .01	50.0 1 .01		48.3 5,856 100
Totals	100 41 .3	100 29 .2	100 11 .1	100 8 .1	100 10 .1	100 3 .02	100 5 .04	100 3 .02	100 4 .03	100 2 .02	100 1 .01	100 12,136 100

[a] In each box the middle set of figures shows the number of tubers which in culture gave the results indicated in the headings of the intersecting columns; the lower right set of figures gives the percentage this number is of the total tubers represented in the horizontal column; and the upper left set gives the percentage of the total tubers in the vertical column.

RELATION BETWEEN ORGANISMS IN SEED POTATOES AND ORGANISMS IN YIELDS

The extent to which organisms present in the seed potatoes were transmitted to the yields as indicated by cultures from the 1917 crop appears in Table IV. Plants grown from seed potatoes from which *Verticillium albo-atrum* was isolated before planting frequently developed wilt (Pl. 140, A) and gave 29.7 per cent of Verticillium infection in the yields. However, not all of the plants grown from the known Verticillium-infected seed were affected by the wilt disease, consequently the percentage of diseased tubers in the yields was considerably lower than it would have been if all the plants had wilted. It was high enough, however, to indicate that infected seed is a very serious factor in carrying the disease from one year to the next. The tubers produced by one lot of rather badly wilted plants showed 40.2 per cent infection with *V. albo-atrum* when cultured, and another lot gave a crop containing 49 per cent infection. The highest percentage of Verticillium infection found in the tubers produced by any considerable number of plants was 92. This result was secured in the yield of 28 plants of the variety Up-to-Date, which is very susceptible to this wilt. In a number of instances individual plants of several different varieties have given yields with 100 per cent Verticillium infection. The average, however, as shown by the records, is nearer 30 to 50 per cent. Some of the plants grown on clean soil from tubers from which either other organisms than *V. albo-atrum* or no organisms were isolated before planting also gave a rather large percentage of Verticillium infection in the yields. For instance, the seed potatoes from which only "miscellaneous fungi" were isolated gave 13.3 per cent Verticillium infection in the yields, and those from which no organisms were isolated gave 18.9 per cent Verticillium infection. This high wilt infection in these two lots apparently came from the spread of the Verticillium fungus from neighboring diseased plants in the field during the growing season, as will be shown more in detail in a later paper.

Fusarium radicicola occurred in only slightly larger amounts in the yields produced from seed potatoes that contained this fungus than it did in those from seed potatoes which according to the cultures made were free from it when planted. For instance, the seed tubers from which *F. radicicola* had been isolated gave back 9 per cent *F. radicicola* infection in the yields, while those from which *F. oxysporum* alone had been isolated gave 8.6 per cent *F. radicicola* infection in the yields, and those from which *Verticillum albo-atrum* alone had been isolated gave 4.4 per cent *F. radicicola.* Practically every lot of tubers cultured during this study has given a noticeable percentage of infection from *F. radicicola* regardless of whether this fungus was proved to be present in the seed potatoes or not. Thus, the indications are that it is transmitted to a slight extent in seed potatoes, that it may come from the soil and is evidently rather commonly distributed. This latter conclusion is in harmony with the work of Pratt (11), who secured this fungus in culture several times from Idaho soils never cropped with potatoes.

TABLE IV.—*Transmission of organisms from seed potatoes to yields in six varieties, season of 1917*[a]

Organisms isolated from seed potatoes.	Number of hills.	Tubers in yield from which were isolated—						Totals.
		Verticillium albo-atrum.	*Fusarium radicicola.*	*Fusarium oxysporum.*	Other Fusarium species.	Miscellaneous fungi.	No organism.	
Verticillium albo-atrum.	151	325 29.7	48 4.4	17 1.6	28 2.6	360 32.9	316 28.9	1,094 100
Fusarium radicicola.	21	3 2.1	13 9.0	5 3.5	2 1.4	67 46.5	54 37.5	144 100
Fusarium oxysporum.	146	101 9.7	89 8.6	39 3.8	19 1.8	346 33.2	447 42.9	1,041 100
Other Fusarium species.	16	8 7.0	3 2.6		5 4.3	20 17.4	79 68.7	115 100
Miscellaneous fungi.	143	131 13.3	39 3.9	4 .4	27 2.7	293 29.7	493 50.0	987 100
None.....	151	204 18.9	42 3.9	17 1.6	31 2.9	317 29.4	466 43.3	1,077 100
Totals.....	628	772 17.3	234 5.3	82 1.8	112 2.5	1,403 31.5	1,855 41.6	4,458 100

[a] In each box the upper set of figures shows the number of tubers which in culture gave the results indicated in the headings of the intersecting columns; and the lower right set of figures gives the percentage this number is of the total tubers represented in the horizontal column.

Similarly *Fusarium oxysporum* occurred somewhat more abundantly in yields grown from known *F. oxysporum* infected tubers than it did in those grown from tubers not known to be infected with this fungus, though in some cases the differences were very slight. For instance, 3.8 per cent *F. oxysporum* infected tubers came from seed known to be infected with *F. oxysporum*, whereas 3.5 per cent, almost as much, came from seed tubers which cultures had indicated to be infected only with *F. radiciola* when planted. It is true with *F. oxysporum* also that in practically every lot of potatoes cultured it was secured from at least a few tubers, even though it was apparently not present in the seed potatoes, indicating that it came from the soil and may be rather commonly distributed. This is in agreement with the findings of other workers (*1*, *6*). The small amount of wilt (Pl. 140, B) caused by this fungus in western Oregon and the low transmission from the seed potatoes to the yields, which was rather unexpected, is thought to indicate that this disease is

not highly virulent in this region. It may, however, be due in part to the apparent general tendency of *F. oxysporum* in the seed potatoes to fail to perpetuate itself in the plants grown therefrom, as shown by the work of Bisby (*1*) and MacMillan (*6*).

Occasionally potato tubers having the stem end sunken and discolored gave both *Fusarium oxysporum* and a small mite in culture. In a few cases both of these organisms were evidently transmitted from the seed potatoes to the yields. For instance, in one hill (Pl. 141, A) grown from a seed tuber from which these two forms were isolated before planting, three out of the nine tubers produced gave the same organisms in culture after harvest. The presence of the mite was no doubt responsible for the sunken condition of the stem end of the invaded tubers, as it naturally would consume some of the tissues and the skin would collapse; and, generally, tubers containing *F. oxysporum* alone appeared quite if not entirely normal on the exterior.

There was a slight tendency of the "other Fusarium species" to be transmitted to the yields, as these organisms ran highest in the tubers grown from similarly infected tubers. Some of these organisms also were found to occur more or less indiscriminately in any lot of tubers cultured.

There was no indication that any of the "miscellaneous fungi" were transmitted to an appreciable extent. They seemed to occur promiscuously with no apparent relation to the condition of the seed potato at planting time.

The seed potatoes from which "no organisms" were isolated gave crops which frequently showed the presence of organisms, doubtless derived from the soil or from adjacent diseased plants, although never to the extent of disease-infected seed.

In order to determine whether or not the stem-end seed piece would consistently transmit a higher percentage of organisms than the eye-end seed piece from the same tubers, a tabulation was made of the available data, the essential points of which are given in Table V. The kind of seed pieces used made no essential difference in the amount of infection from *Verticillium albo-atrum* secured either in the plants produced or in the yields obtained, the percentage of infection in the yields from the stem pieces being 22.7 and from the eyepieces 24.6. This difference is entirely negligible, being even less than the difference in the results secured from planting the two longitudinal halves of other tubers which presumably should have shown no difference in amounts of infection in the yields. However, in view of evidence later secured on the spread of *V. albo-atrum* from one plant to another in the row, the results presented here on the transmission of this organism in the different kinds of seed pieces must not be given too great weight, because the plants from the different kinds of seed pieces were grown in juxtaposition in the row, thus giving the fungus opportunity to spread from one plant to another.

Nevertheless, the results so far as they go are in agreement with the findings of Pethybridge (*9*) in Ireland, who has demonstrated that this wilt fungus is not localized at or near the stem end of affected tubers. There is, therefore, no particular value in the attempt to avoid Verticillium-wilt in seed potatoes by discarding the stem ends and planting only the eye ends of tubers suspected of containing this wilt fungus. Reliance for the control of this disease should rather be placed on other methods which have been demonstrated to be effective.

TABLE V.—*Transmission of organisms from different portions of the same seed tubers and from whole seed tubers to the tubers yielded by six varieties, season of 1917*

Organism isolated from seed potato.	Number of seed pieces planted.	Percentage of same organisms in yields as was present in the seed potato according to kind of seed piece used.				
		Stem pieces.	Eye pieces.	One longitudinal half.	Other longitudinal half.	Whole tubers.
Verticillium albo-atrum..	151	22.7	24.6	17.4	20.5	35
Fusarium radicicola.....	21	12.9	4.3	4.5	5.9	14.7
Fusarium oxysporum....	146	2.9	2.3	7.7	5.8	3.8

Fusarium radicicola occurred noticeably more in the yields from the stem pieces than from the eyepieces, 12.9 per cent and 4.3 per cent, respectively. The number of seed pieces planted containing this fungus were so few that definite conclusions can not be drawn, and the differences noted might not hold true in an examination of a larger number of cases.

Fusarium oxysporum was transmitted to such a small extent in any of the potatoes experimented with that it is difficult to judge whether the stem-end pieces tend generally to give more disease than the eye-end pieces. A few tubers, though, apparently gave striking evidence of this tendency (Pl. 139, C). For instance, one infected tuber gave 75 per cent infection in the yield from the stem-piece plant and no infection in that from the eye-piece plant; another gave 33 and 14 per cent, and another gave 10 and 0 per cent, respectively. The average results, however, do not point strongly in this direction, and it is apparently true with this organism also that the practice of discarding the stem ends of seed potatoes is not a reliable method for the control of the disease and ought to be abandoned in favor of other more effective methods.

DISTRIBUTION AND IMPORTANCE OF VERTICILLIUM ALBO-ATRUM, FUSARIUM OXYSPORUM, AND FUSARIUM RADICICOLA IN OREGON

Although there has been no opportunity to make a systematic survey of these potato diseases in the entire State of Oregon, a number of scattered collections have been made in different regions and many specimens have been sent in by growers and by county agricultural agents. There remain sections, however, where potatoes are grown and

from which no specimens have been secured. The data presented here must, therefore, be considered incomplete. The status of these diseases is better known in western Oregon, the region west of the Cascade Mountains, than it is in the central and eastern portions.

Verticillium albo-atrum has been found in 16 counties, which embrace most of the important potato-growing areas of the State (fig. 5). It is widely distributed and of common occurrence at least in western Oregon, and every season causes an appreciable loss in many fields. The highest percentage of plants wilted from this fungus found in any commercial field was 30. The more usual amounts found are from 5 to 7 per cent. In the work on certification of potatoes in 1918, Verticillium-wilt was

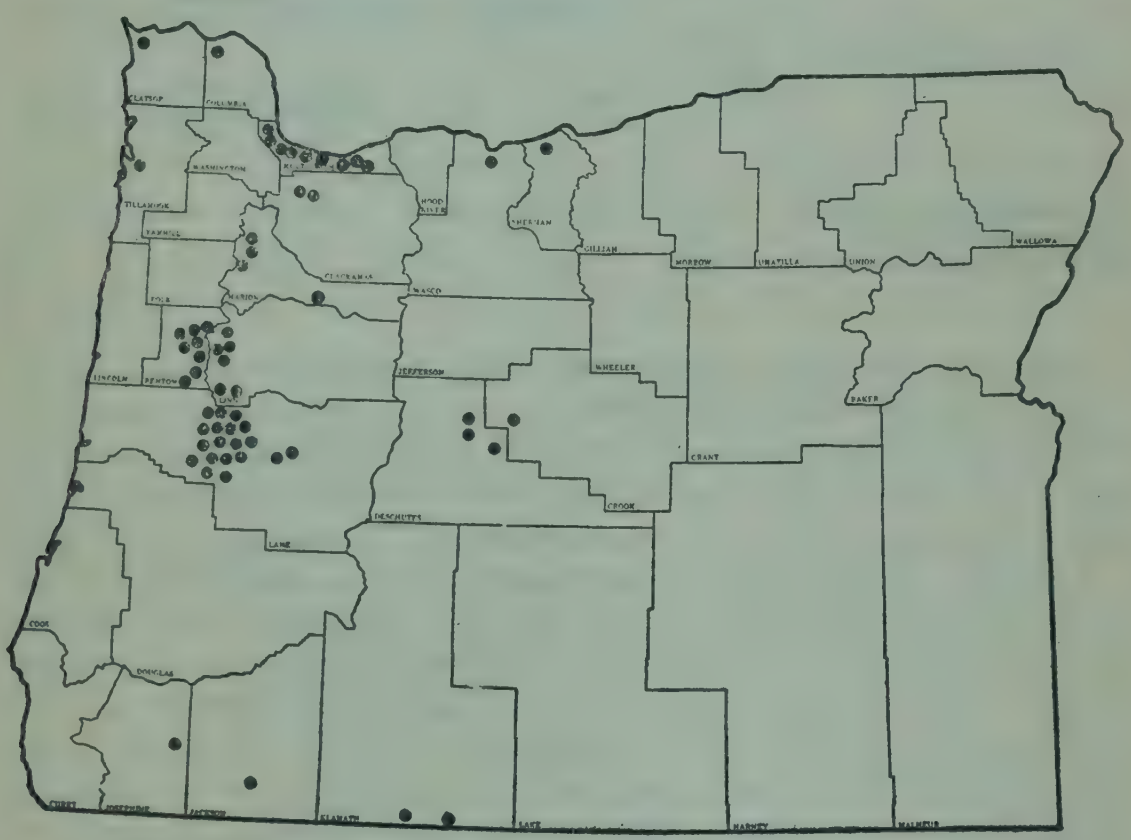

FIG. 5.—Map of Oregon, showing the localities where *Verticillium albo-atrum* has been collected on potatoes during the past few years.

the second factor in the order of importance in causing the failure of fields entered to pass the inspections. In this certification work or in any seed plot improvement work it is very necessary, therefore, to give due consideration to the nature of the disease and to the ways in which it is disseminated.

The yields of plants affected with Verticillium-wilt are usually greatly reduced, which may be indicated by a few specific examples.

Two plots of potatoes consisting of 73 hills each were grown in 1917 to test the influence of Verticillium-wilt on the yields produced. The seed tubers used were produced by apparently wilt-free plants and gave *Verticillium albo-atrum* in no case in cultures made before planting. They were each cut in two lengthwise, one half being planted in one plot

and the other half in the other plot, so that the seed employed was presumably quite disease-free and was as nearly identical in the two plots as possible. In one plot the seed pieces were inoculated by placing on top of each a culture of *V. ablo-atrum* growing on sweet clover stems before covering them with soil in the furrow, and the other plot was left uninoculated as a control. The plots were located a short distance apart on the same kind of soil on which the crops had been well rotated and which had not grown potatoes for at least five years. Ninety-nine per cent of the plants on the inoculated plot were affected by *V. albo-atrum* before the

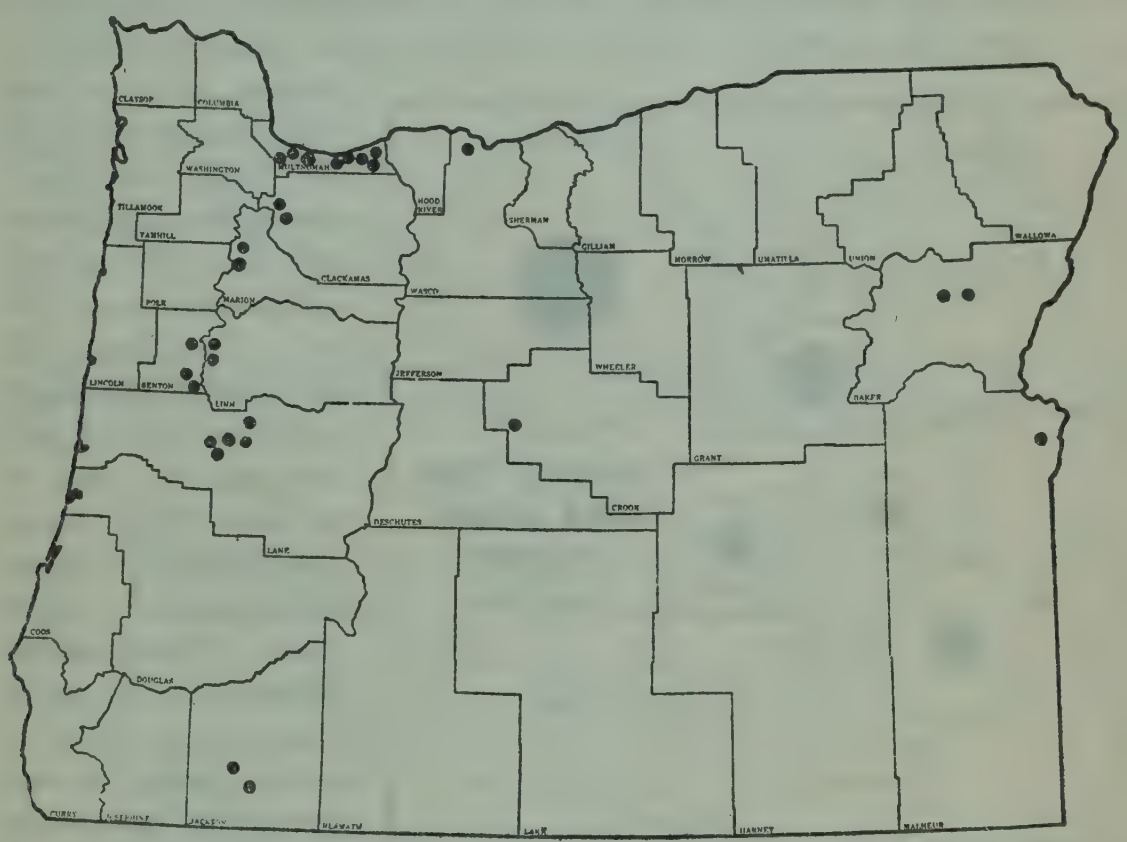

FIG. 6.—Map of Oregon, showing the localities where *Fusarium oxysporum* has been collected on potatoes during the past few years.

end of the season, and none of those on the control plot were found wilted. The yield on the diseased plot was 31 per cent less than that on the control plot.

In 1919 two ¼-acre plots were tested similarly. The seed used came from the same lot, but the tubers were not, as in the previous trial, cut longitudinally into halves and planted one in each plot. In this test there was a 59 per cent lower yield on the diseased than on the healthy plot. If the disease attacks the plants early in their growth the yield is usually greatly reduced, but if the attack comes late in the season the crop is practically normal in amount, though often a high percentage of the tubers carry the disease. The average reduction of yield in diseased plants probably ranges from 30 to 40 per cent.

Fusarium oxysporum has been found in 11 counties of Oregon in widely separated localities (fig. 6), though much less frequently then *Verticillium*

albo-atrum. In most cases it has been encountered attacking only scattering plants in the field and does not cause nearly so much damage as the other wilt organism. Only two fields—one in Lane County and the other in Multnomah County, both of western Oregon—have so far been reported to or seen by the writer where this fungus was recognized as causing a high percentage of disease. In the former field about 10 per cent and in the latter about 30 per cent of the plants were affected. The reason for these high percentages of infection in these two fields was not apparent.

One test was made at Corvallis in 1917, to determine the influence of this disease on the yield. One lot of seed potatoes was inoculated at planting time in the same manner as in the test with *Verticillium albo-atrum* noted above and the other was left uninoculated as a control. The disease did not develop sufficiently in the inoculated plot to reduce the yield in the least, which was in considerable contrast to the action of *V. albo-atrum.* For where this fungus was used 99 per cent of the plants wilted during the season and 49 per cent of the tubers were infected by the same fungus when cultured after harvest, but where *Fusarium oxysporum* was used less than 5 per cent of the plants showed wilt and only 6 per cent of the tubers were infected by this same fungus when harvested. According to all available information this disease must be considered of relatively minor importance in western Oregon. The data at hand do not justify any definite conclusions as to its importance in the eastern part of the State, where it may prove to be more serious.

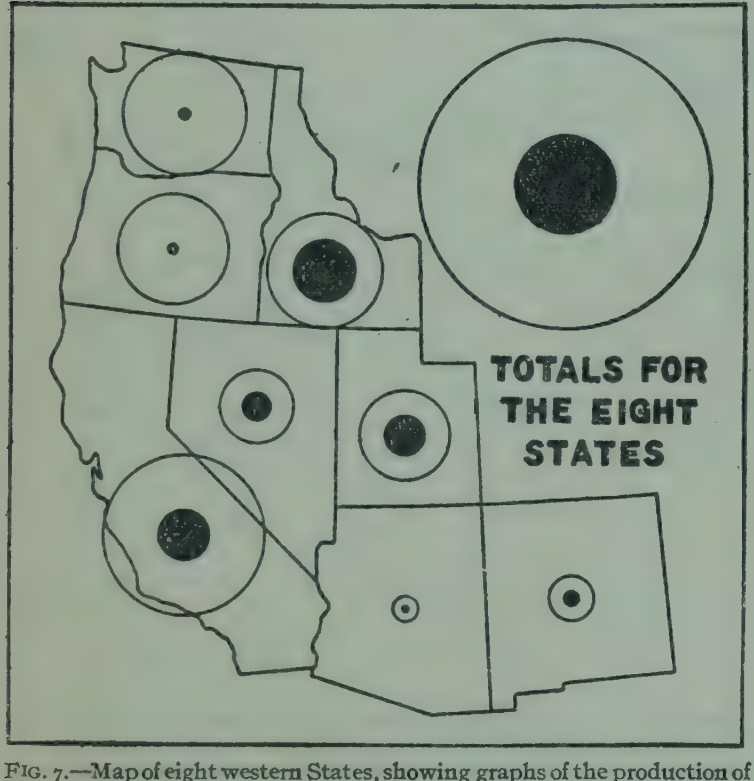

FIG. 7.—Map of eight western States, showing graphs of the production of potatoes in the different States in 1917 and the losses caused from wilt due to *Fusarium oxysporum.* The black area of the graph shows the loss from Fusarium-wilt; the white area, the production of potatoes; and the entire area, the production of potatoes if there had been no loss from Fusarium-wilt.

There are areas in the Pacific coast and mountain States where the Fusarium-wilt is without doubt a factor of considerable importance in potato growing. The Plant Disease Survey (*12*) has published estimates showing losses in these States in 1917, ranging from 1 to 30 per cent and

averaging 14 per cent (Table VI and fig. 7). The larger losses in this area occur in general in the hotter, irrigated sections. Oregon is included with an estimated loss of about 1 per cent.

TABLE VI.—*Effect of Fusarium-wilt on the yield of potatoes in the western United States in 1917 (12)*

State.	Production in 1917.	Estimated loss from Fusarium-wilt.	
	Bushels.	*Bushels.*	*Per cent.*
Washington	9,875,000	100,000	1
Oregon	8,100,000	82,000	1
California	15,225,000	1,691,000	10
Idaho	6,084,000	2,607,000	30
Nevada	3,105,000	548,000	15
Utah	4,347,000	1,087,000	20
Arizona	420,000	47,000	10
New Mexico	1,276,000	174,000	12
Total	48,432,000	6,336,000	[a] 14

[a] Average.

Fusarium radicicola has been secured from potatoes in nine counties of the State (fig. 8). It, too, is seemingly of common occurrence, but is

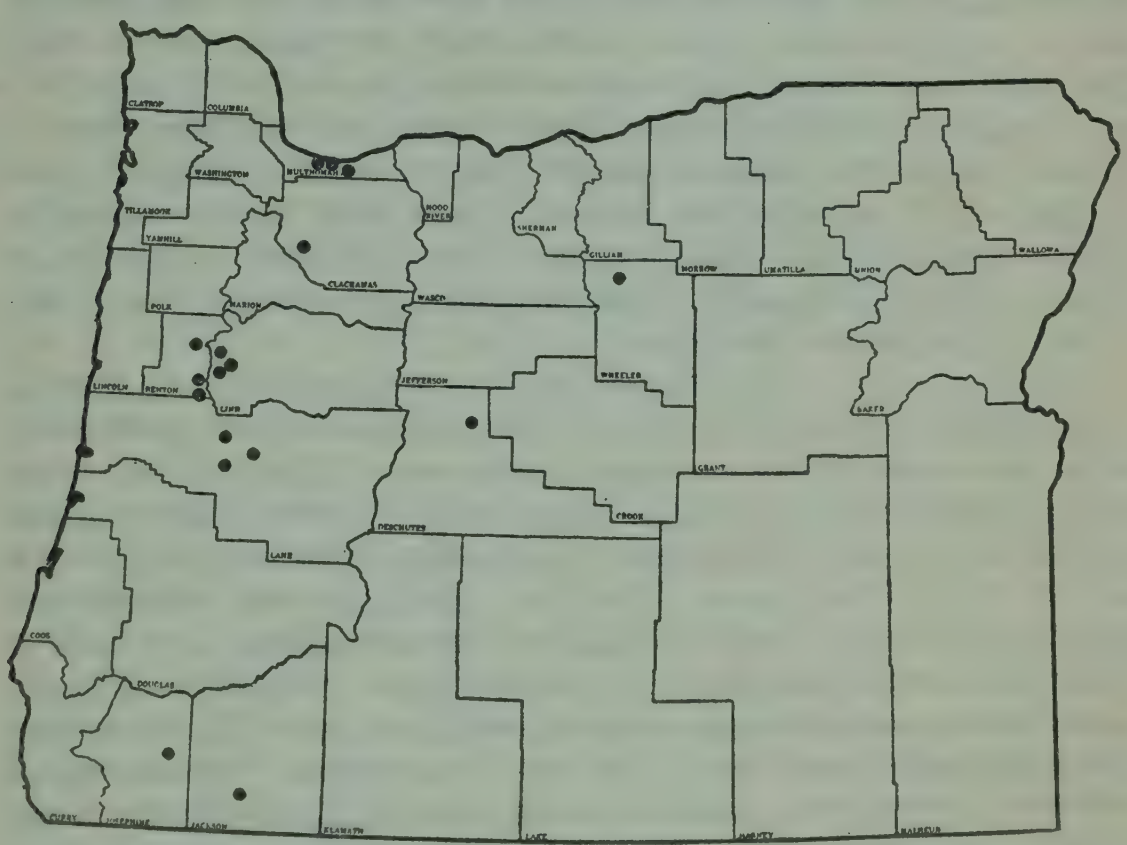

FIG. 8—Map of Oregon, showing the localities where *Fusarium radicicola* has been collected on potatoes during the past few years.

apparently of little importance to potato production here. It has not been reported as a cause of wilt in other places and evidently does not often cause any wilt of the plants in Oregon, though the fact that it has

occasionally been isolated in pure cultures from discolored stem tissues of wilted plants suggests that it may occasionally cause a wilt of potato plants. It has been secured most frequently from tubers which were perfectly sound except for the presence of discoloration in the stem-end vascular region. For instance, 82.4 per cent of the tubers which gave this organism in cultures were browned or yellowed in this region (Table I) and were not in any way affected by rot. Only 8.3 per cent of the tubers which gave this fungus in culture were affected by stem-end rot of some form or other, varying from a dry, withered rot to a moist, soft, and light brown colored rot more typical of the condition known as "jelly end" rot. The specific black fieldrot of potato tubers due to *F. radicicola* as stated to occur in Idaho (*10*) and eastern Washington (*5*) has not yet been reported to or seen by the writer in Oregon. The conditions in the eastern part of the State remain largely, however, still to be investigated.

A stem-end rot of potato tubers occurs not infrequently during some seasons in western Oregon, and to some extent in the rest of the State, though in other seasons it is rarely encountered. It affects only the long tubers and varies widely in type and extent of rot at harvest time from a mere withering unaccompanied by discoloration of the stem end of the tuber as though a part of the water had been withdrawn from that portion, to a dry, wrinkled, sunken, rather tough, and light brown to black discolored condition of ½ inch or more of the stem end of the tuber, or to a soft and rather jellylike light brown colored rot extending back at times 1½ inches from the stem end, the rest of the tuber being sound and unaffected. In storage these diseased tubers often do not rot further, but the affected tissues frequently dry down and form a sharp line of demarcation from the sound, unaffected tissues unless stored under conditions unfavorable to the tubers.

The cause of this rot in the State and the relationship of the different types manifested are not known, as it has been studied only incidentally when encountered in the course of other work. Generally the tubers appear similar to if not identical with the jelly endrot or the stem-end dryrot as described by Carpenter (*2*) of which *Fusarium radicicola* was said to be the chief cause. However, this fungus was isolated from only 12.2 per cent of the stem-end rot tubers cultured and listed in Table I, while the majority, 53.9 per cent, gave "miscellaneous fungi" which were presumably saprophytic in most cases, and 8.8 per cent gave *Verticillum albo-atrum* and 4.5 per cent *F. oxysporum*. Tubers affected by this type of rot are, of course, readily entered by saprophytic organisms, and in many cases it is difficult if not impossible to determine from isolation cultures the primary cause of the trouble. However, the large variety of organisms secured in culture from affected tubers, the occurrence of the trouble only during certain seasons without any apparent relation to the condition of the seed potatoes at planting time or to crop rotation, and the presence of *F. radicicola* in the stem end of so many

tubers not affected by rot lead one to suspect that the appearance of the trouble depends more on climatic conditions than it does on the presence of any one of two or three organisms.

It seems possible and perhaps probable that when moisture in the soil is deficient in the latter part of the growing season, as it frequently is in western Oregon, and the transpiration of the plant is relatively excessive during hot weather the plant might, as sometimes occurs in fruits (*3*), actually withdraw water from the stem end of the tuber, giving a sunken withered condition favorable to the entrance of various organisms both parasitic and saprophytic. There might then be a gradual progress from this condition to typical jelly endrot, and in each case the condition of the tuber at harvest time would perhaps depend on the particular conditions surrounding it during growth and the period of maturing. It remains, however, for future work to determine what the facts in the case are. It should be stated that for the present no special measures for the control of this stem-end rot can be definitely recommended.

SUMMARY

The studies reported here were conducted in western Oregon, and the results apply to western Oregon conditions. They may not apply in their entirety to the rest of the State.

In western Oregon, *Verticillium albo-atrum* is more important than *Fusarium oxysporum* as a cause of wilt of potato plants. Each of these organisms is frequently present in the stem-end vascular region of potato tubers produced by diseased plants, and their presence there is usually though by no means always indicated by distinct discoloration of that region. An average of 6.6 per cent and occasionally as high as 14.4 per cent of the tubers which gave *V. albo-atrum* in culture were not discolored.

Fusarium radicicola also is often present in this same region of the potato tuber, though it is not known to be a cause of wilt of the plants; and the discoloration generally accompanying it cannot be distinguished from that usually present with the two common wilt-producing organisms, *Verticillium albo-atrum* and *F. oxysporum*.

Fusarium radicicola caused heavier discoloration than *Verticillium albo-atrum* in the infected tubers, while *F. oxysporum* caused still heavier discoloration than either of the other two organisms.

In addition to these three fungi there are also several others that invade the stem-end vascular region of potato tubers, and the longer the potatoes are kept in storage the larger is the number of tubers which give organisms in culture. Some of these are parasitic on potatoes and many others are not, so far as known, associated in any way with a disease of this crop. They are usually though not always accompanied by discoloration of the tuber tissues.

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The presence of discoloration in the stem-end vascular region of potato tubers is not a trustworthy index of the presence of disease-producing organisms therein and ought not to be relied upon exclusively as a guide for the separation of diseased from healthy tubers for planting purposes. For instance, 45 per cent of the tubers which were "browned" in the vascular region when cultured gave organisms which cause disease in potatoes, and 55 per cent gave either no organism or miscellaneous fungi of no apparent importance; 22 per cent and 5 per cent of those which were "yellowed" and "not discolored," respectively, gave organisms parasitic on potatoes, the others of these lots giving nothing of consequence.

Verticillium albo-atrum occurred in all sizes of potato tubers, though somewhat more extensively in the small ones than in those of medium size. *Fusarium oxysporum* and *F. radicicola* did not seem to occur considerably more in one size of potato tuber than in another. The medium-sized tubers gave a larger proportion of miscellaneous organisms than the smaller ones.

Verticillium albo-atrum is transmitted to a considerable extent from seed potatoes to yields. From 30 to 50 per cent of the tubers grown from known infected seed potatoes were invaded by the same organism when harvested and cultured. *Fusarium radicicola* and *F. oxysporum* were transmitted to only a slight extent from seed potatoes to yields and were apparently not infrequently present in soils which had not been planted to potatoes for several years.

The stem-end seed pieces did not seem to give more disease either in the plants or in the tubers produced therefrom than the eye-end seed pieces of the same infected tubers. The differences are at least so slight that it apparently is not advisable in attempting to avoid wilt in seed potatoes to rely much on the practice of discarding the stem ends and planting only the eye ends of tubers suspected of containing wilt organisms.

Verticillium albo-atrum is widely distributed in Oregon, having been recorded from 16 counties, and is the most important potato wilt-producing organism in the western part of the State. It causes appreciable losses each year, not only in commercial fields but especially in seed plots, because the presence of this fungus greatly lessens the value of the potatoes for seed purposes. It annually is responsible for the failure of a considerable number of fields to pass inspection for certification. The yields of affected plants are reduced on the average of from 30 to 50 per cent.

Fusarium oxysporum is also widely distributed in the State, having been noted in 11 counties, though it is seemingly of little importance, at least in the western portion. In only a few cases have any considerable number of plants been found attacked by this organism in any western Oregon fields examined.

Fusarium radicicola occurs frequently in potato tubers in Oregon, though it usually does not produce any apparent ill effects. It is often associated with a slight and generally rather dry, withered endrot or a soft and moist endrot, but it is not believed to be the sole cause of this trouble. It is more often found in tubers that are sound, except for having discolored vascular regions near the stem end.

A stem-end rot of potato tubers, varying from a mere withering of the stem end to a dry, withered, brown to black rot, or to a soft jellylike light brown rot, occurs rather commonly during some seasons in western Oregon. It appears to be due as much to climatic conditions during the growing season as to the presence of any certain parasitic organisms.

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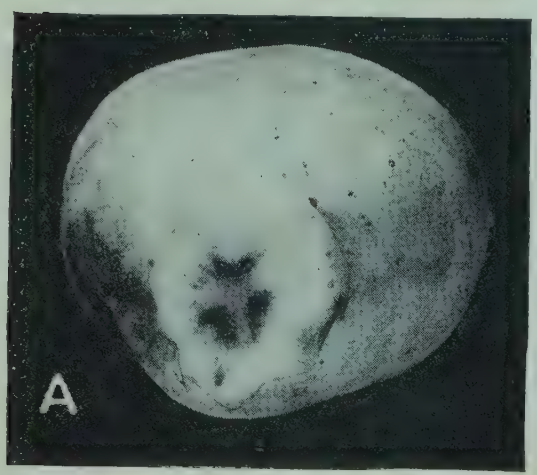
PLATE 139

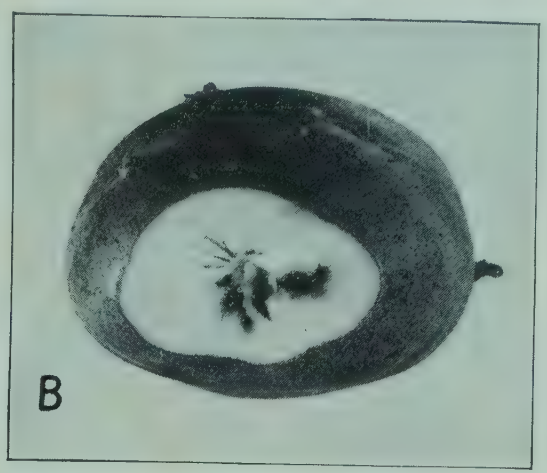
A.—Up-to-Date potato tuber with surface of stem end cut off to show discolored vascular tissue from which *Verticillium albo-atrum* was isolated in culture.

B.—Early Rose tuber from which *Fusarium oxysporum* was isolated.

On the average *F. oxysporum* caused heavier discoloration than did *V. albo-atrum* in the invaded tubers, though the appearance of the tubers could not be depended on for differentiating between the two organisms.

C.—Early Rose potato tuber from the discolored portion of which *F. oxysporum* was isolated in culture. The two plants grown from the two stem-end seed pieces became diseased, did not grow more than 9 inches in height, and died without producing any yield. The two plants from the two eye-end seed pieces remained healthy throughout the season and produced an average yield of 45 ounces. This difference in results from the stem-end and eye-end seed pieces is unusual, however, for on the average the former performed almost as well as the latter.


A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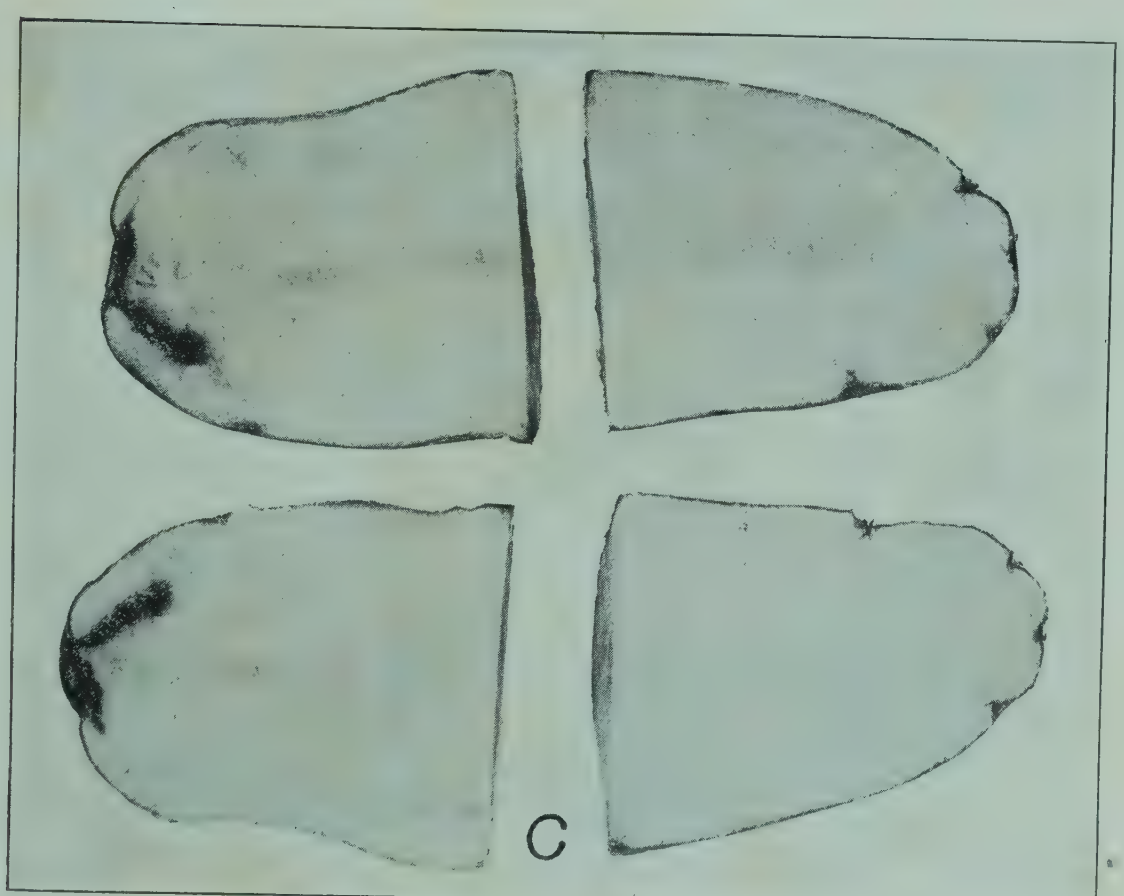
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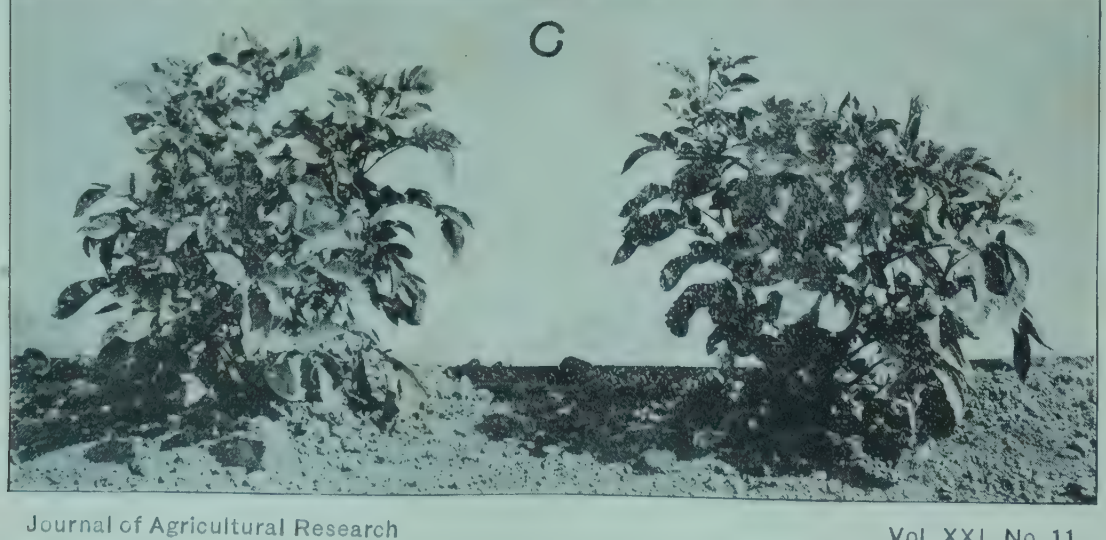
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PLATE 140

Influence of the presence of wilt-producing fungi in seed potato tubers on the plants grown therefrom. All plants photographed August 22, 1917.

A.—Plant at left wilting and dying prematurely from attack of *Verticillium albo-atrum* demonstrated to be present in the seed potato before planting. Plant at right was grown from a tuber from which no organism was secured in culture. The two seed potatoes each weighed 2 ounces, and it is interesting to note that both were produced by one plant affected by Verticillium-wilt. Variety Up-to-Date.

B.—Two American Wonder potato plants grown from the stem-end (plant at left) and eye-end (plant at right) halves of a 4-ounce tuber from which *Fusarium oxysporum* was isolated before planting. Neither of the plants showed wilt during the season and none of the tubers were infected by this fungus when cultured after harvest. The contrast between the amounts of disease in the plants grown from tubers affected by *F. oxysporum* and by *V. albo-atrum* was striking, for so few of the former became noticeably attacked. The latter, on the other hand, were generally wilted.

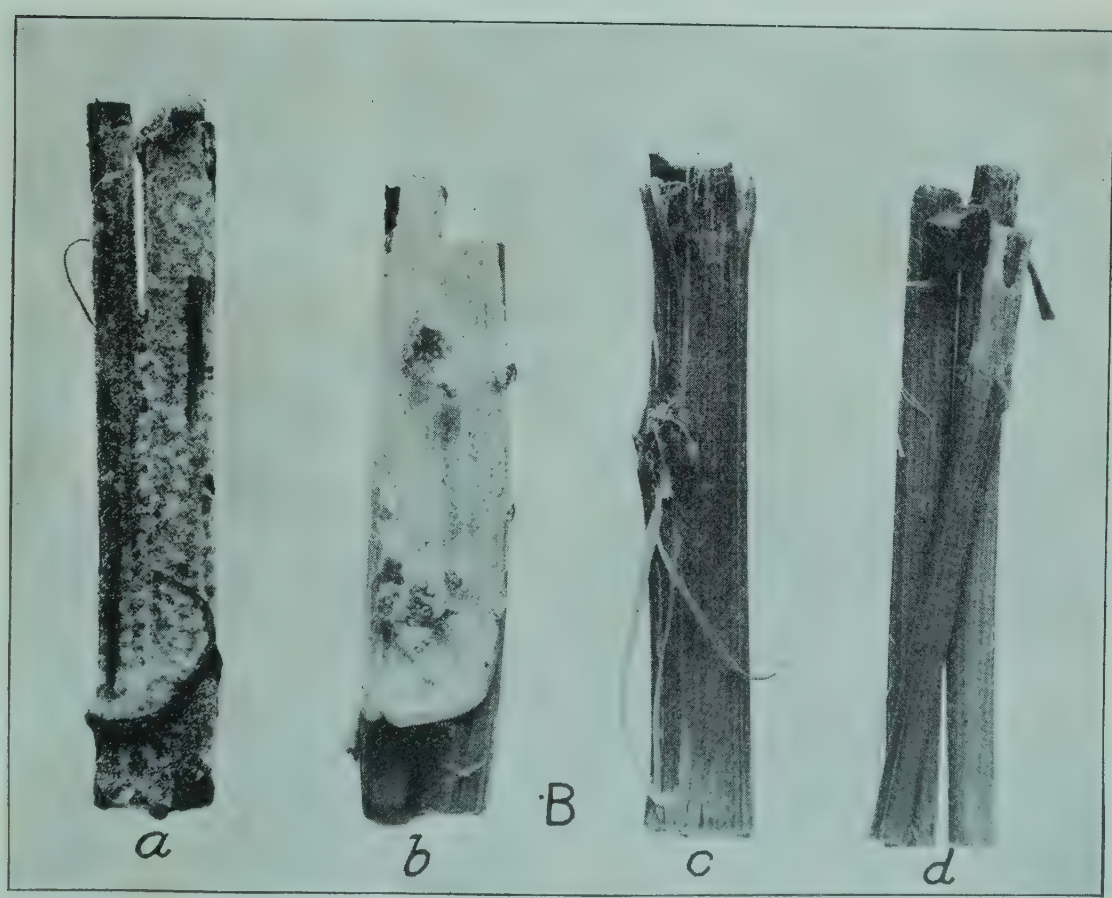
C.—Two American Wonder potato plants grown from the two longitudinal halves of a 5-ounce tuber from which no organism was isolated in culture. No wilt developed during the season.

PLATE 141

A.—Yield of potato tubers grown from a seed potato which had a slightly withered and collapsed area at the stem end from which *Fusarium oxysporum* and a small mite were secured in cultures. Three of the tubers in this yield were affected in the same way as the seed tuber and gave the same results in cultures. The sunken condition of the stem end of these three tubers was probably due to the presence of the mite and is not typical of tubers invaded by *F. oxysporum* alone. In this latter case the tubers generally appear normal on the exterior.

B.—Original isolation cultures (77 days old) of wilt-producing organisms secured on sweet clover stems from the four quarters of the stem end of one Up-to-Date potato tuber: *a*, *Verticillium albo-atrum; b*, *Fusarium oxysporum; c*, *d*, no growth. At the top of *d* is shown the piece of tissue removed from the tuber for inoculating the clover stem.

A


a
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B
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d

CORRELATION AND GROWTH IN THE BRANCHES OF YOUNG PEAR TREES [1]

By H. S. REED [2]
Professor of Plant Physiology, College of Agriculture, University of California

The present paper attempts to make a quantitative study of the relationships of laterals produced by the young branches of the pear tree. Such an inquiry should be of value both to the physiologist who is interested in the mechanism of development and to the horticulturist who is interested in the production of a tree of a given type.

In a previous paper (*10*)[3] it was shown that upright young pear shoots tend to remain unbranched so long as the apical bud grows uninterruptedly. If the apical bud is arrested in its development, or is removed, a neighboring lateral bud promptly develops and continues the lineal growth of that member. Moreover, if the apical portion of a shoot is amputated, the buds immediately below the point of amputation are not only the first to develop into shoots but produce the longest shoots.

A plantation of young Bartlett pear trees (*Pyrus communis* L.) growing at the Citrus Experiment Station, Riverside, Calif., furnished the data upon which this study is based. The trees were planted in the orchard in 1916, and the measurements were taken in February, 1920. The lateral shoots (Pl. 142), hereafter designated as "laterals," grew during the summer of 1919, and the mother shoots on which they were borne grew in the summer of 1918.

During the early life of the orchard, certain plots of the trees were systematically "headed back" to form a framework of a particular type. Accordingly these plots of trees were pruned in the winter of 1918–19 by amputating from one-half to three-fourths of all of the principal upright shoots grown in the preceding season. The portion remaining after pruning is designated as the "mother shoot" in the following discussion. The unpruned trees never received any pruning after the first year. The lateral shoots grew on the mother shoots during the summer of 1919. The relations of the two will be readily comprehended upon referring to Plate 142.

The mother shoots used in this study stood in an approximately vertical position and on the small trees were in no way crowded. The results are not, therefore, believed to be greatly influenced by differences

[1] Paper No. 78, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[2] The writer wishes to mention his indebtedness to Mr. F. F. Halma for assistance in the calculations and to Dr. J. A. Harris for valuable suggestions.

[3] Reference is made by number (italic) to "Literature cited," p. 874–875.

in illumination. None of the trees had produced any fruit up to the time these measurements were made.

The two classes of mother shoots (pruned and unpruned) were on different trees. The 270 pruned mother shoots were on 91 trees which had received systematic "heading back"; the 54 unpruned mother shoots were on 25 trees which received no pruning. While this distribution does away with a certain closeness of spatial association, it prevents the possible transmission of influences from one class of mother shoots to the other.

In the present paper the term "growth" is used to designate a permanent increase in size. It is well recognized, however, that the same term may also be applied to phenomena in which there is little if any increase in size—for example, differentiation of organs and tissues.

The length of the lateral was taken as a simple measure of its growth. The almost complete absence of secondary laterals of any consequence allows this kind of measurement to be taken with a comparatively small error. Even where secondary laterals were produced there was no evidence that they had limited the length-growth of the primary lateral.

The shoots produced in a single season on a vigorous pear branch are usually of two kinds—"laterals" and "fruit spurs." The general characteristics of these two types of shoots have been discussed by Vöchting (*14*) and others. While it is convenient to consider the two types of shoots separately, it is necessary to remind the reader that their differences are primarily quantitative and not qualitative. The fruit spurs ordinarily attain but a short length and produce fruit-bearing buds. Under various circumstances they may, however, be forced to make strong vegetative growth and become "laterals." Such results often follow severe pruning or a change of position of the parent shoot whereby the dorsally placed "fruit spurs" will develop into vegetative "laterals" (*10*). A true dimorphism does not seem to exist in the young branches of the pear tree.

The "first" lateral is designated as the lateral at the distal end of the mother shoot, and the others follow in serial order toward the proximal end of the mother shoot. The "original" number of buds on a mother shoot was the number present before any laterals had grown—that is, at the beginning of the 1919 growing season.

The mother shoots on the pruned trees had a mean length of 44.1 cm. and a coefficient of variability of 39.2 per cent. The original number of buds on these mother shoots had a mean of 15.5 buds with a coefficient of variability of 41.1, and so elsewhere. The mother shoots on the unpruned trees had a mean length of 50.6 cm. with a coefficient of variability of 29. The number of original buds they possessed was 21.5 with a coefficient of variability of 25.2. The frequency distributions of these variants are shown in figure 1. Both distributions are asymmetrical and have a skewness in the direction of the higher values.

I. GROWTH AND DIFFERENTIATION OF LATERALS

The characteristic growth of laterals and fruit spurs on the mother shoots is to be regarded as an expression of their capacity to develop under the influence of the various internal and external factors at work. An analysis of their nature and variability ought to throw some light upon the laws of growth.

Such an analysis will involve an investigation of at least two phases of the laws of growth—first, the total quantity of shoot wood produced upon the mother shoots, and secondly, the differentiation found in the laterals produced upon the mother shoot. At all stages of the inquiry

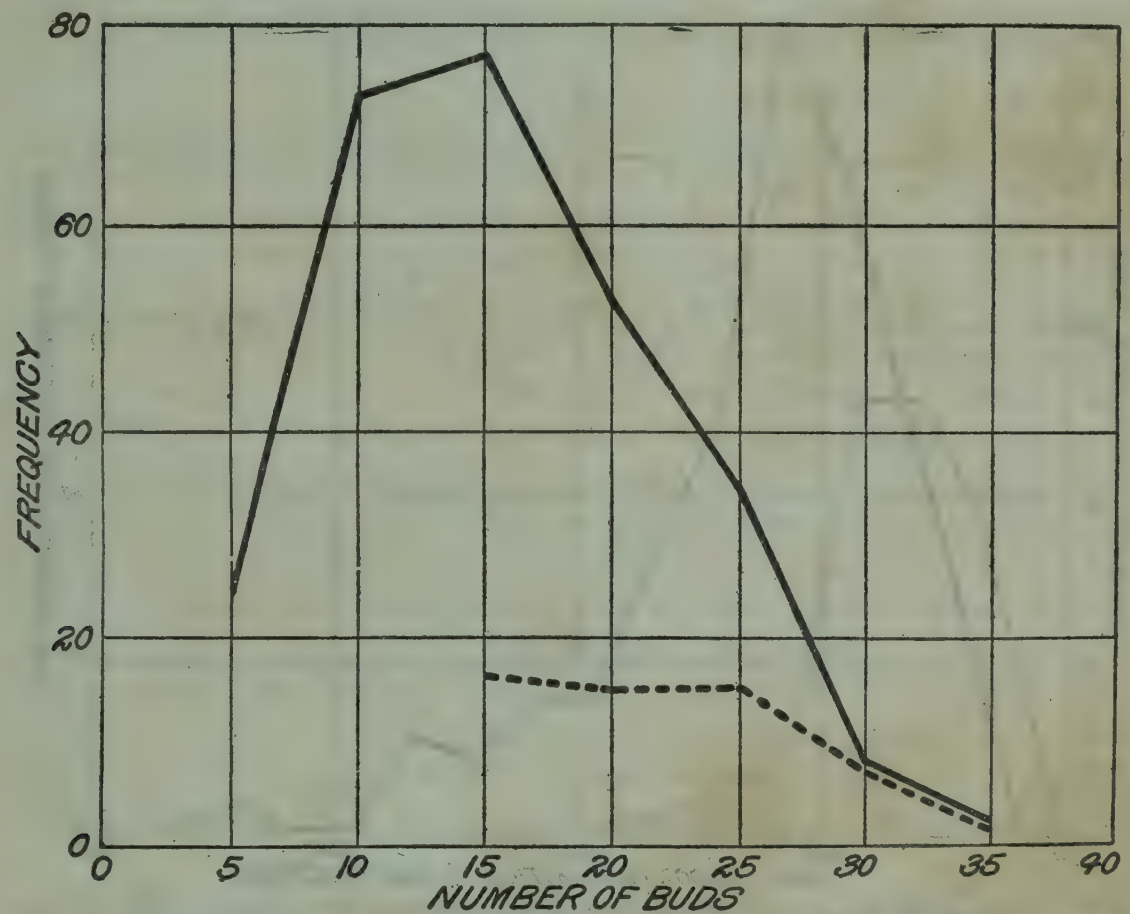

FIG 1.—Frequency distributions of buds on mother shoots. The solid line indicates distribution on pruned shoots and the broken line distribution on unpruned shoots.

it will be profitable to compare the growth produced on the pruned mother shoots with that produced upon mother shoots of similar age which received no pruning.

It will doubtless seem to some readers that the investigation treats the mother shoots to too great a degree as independent organisms. The only reply to such a criticism is that it seemed impossible to treat them in any other way. While correlative influences undoubtedly exist between the different mother shoots, it seems at present impossible to evaluate them. By using a sufficiently large population, it is probable that such correlated influences are to a large extent equalized. It may be stated, however, that the upright shoots on young pear trees are sufficiently uniform for all purposes of the present investigation.

TOTAL PRODUCTION OF LATERALS ON THE MOTHER SHOOTS

Reference to Tables I and II gives a general idea of the total length of all laterals produced upon the 324 mother shoots measured.[1] It is at once evident that there is pronounced variability in the total amount of new wood produced by individual mother shoots. The mother shoots which had been pruned in the preceding winter produced an average total growth of 251.5±4.9 cm. The total shoot production of the pruned mother shoots ranged from 85 cm. to 685 cm., with an interquartile range of 148.53 cm. and a coefficient of variability of 44.91 per cent. The unpruned mother shoots produced an average total growth

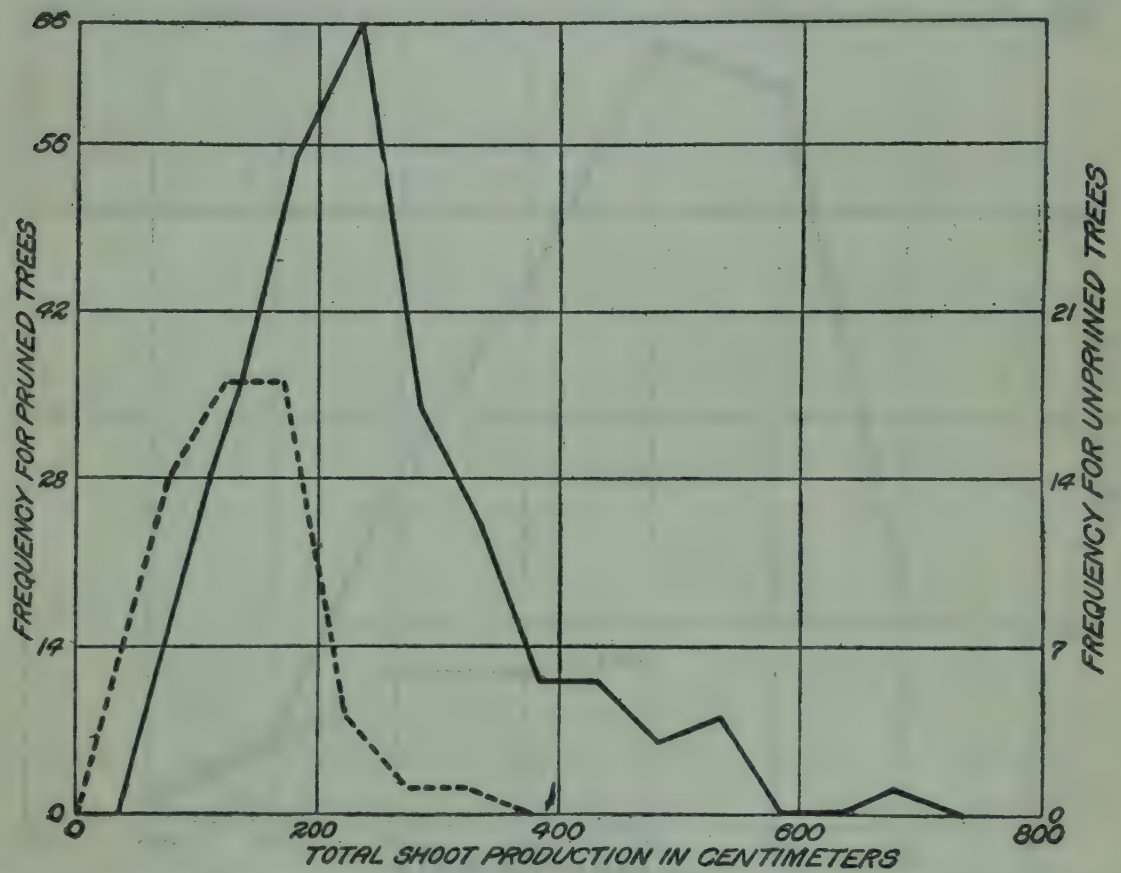

FIG. 2.—Frequency distributions of total shoot growth on mother shoots. The solid line indicates distribution on pruned shoots and the broken line distribution on unpruned shoots.

of 140.5±5.3 cm. The total length of new shoots on unpruned mother shoots ranged from 75 cm. to 325 cm., with an interquartile range of 71.44 cm. and a coefficient of variability of 37.82 per cent. So far as these figures go, there is, therefore, slightly more variability in the total amount of new wood produced by the pruned mother shoots. The graphs in figure 2 show the nature of the two populations when the quantity of new wood produced is used as abscissa and the frequency as ordinate. The class range for the growth on the pruned shoots was 60 cm. and that for the growth on the unpruned shoots was 50 cm.

[1] The presentation of the actual measurements of the laterals would fill several very large tables. While I realize that these measurements should be published, it seems best in the interests of economy to summarize the data and give them in Tables I and II. The complete data are on file at the Citrus Experiment Station, and copies will be sent to workers who feel that they might profit by them.

TABLE I.—*Mean length of laterals on pruned mother shoots*

Lateral No.	On mother shoots which, after pruning, had—								
	4 to 6 buds.	7 to 9 buds.	10 to 12 buds.	13 to 15 buds.	16 to 18 buds.	19 to 21 buds.	22 to 24 buds.	25 to 27 buds.	28 to 37 buds.
	Cm.	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*
I	126.6	121.2	114.5	110.2	110.5	103.6	101.4	97.7	97.2
II	73.6	71.4	69.6	68.3	60.2	70.9	72.1	76.6	73.3
III	38.7	42.2	31.8	33.5	29.2	22.1	29.3	31.1	16.5
IV	6.6	13.6	9.7	14.2	13.1	9.9	9.3	10.4	.3
V	.8	17.5	6.7	6.9	6.2	10.1	8.4	15.7	13.4
VI	0	1.7	.5	2.1	1.9	1.0	3.8	3.7	3.5
VII	.....	1.7	3.8	1.3	4.6	1.5	6.1	5.1	2.4
VIII	.....	.03	2.6	2.8	3.1	1.7	.7	3.3	.3
IX	.....	0	.2	.2	1.8	2.8	.3	1.2	1.1
X	.....	.....	.8	2.3	.5	1.3	1.7	.1	2.5
XI	.....	.....	.5	1.9	2.0	2.2	3.7	6.0	1.9
XII	.....	.....	0	.7	1.2	2.0	.4	.9	11.5
XIII	.....	.....	.....	1.5	.9	2.7	.8	3.1	.9
XIV	.....	.....	.....	0	.8	.3	.7	8.1	.8
XV	.....	.....	.....	0	.2	1.3	.3	1.1	2.3
XVI	.....	.....	.....	.....	1.6	1.1	.3	7.1	1.9
XVII	.....	.....	.....	.....	0	1.3	1.4	8.4	11.8
XVIII	.....	.....	.....	.....	0	.1	1.6	3.7	3.1
XIX	.....	.....	.....	.....	.....	1.3	3.4	2.2	7.6
XX	.....	.....	.....	.....	.....	0	.2	.4	.9
XXI	.....	.....	.....	.....	.....	0	.2	2.5	2.0
XXII	.....	.....	.....	.....	.....	.....	0	1.6	9.5
XXIII	.....	.....	.....	.....	.....	.....	0	9.9	.4
XXIV	.....	.....	.....	.....	.....	.....	0	9.7	7.4
XXV	.....	.....	.....	.....	.....	.....	.....	.2	6.6
XXVI	.....	.....	.....	.....	.....	.....	.....	3.3	.4
XXVII	.....	.....	.....	.....	.....	.....	.....	0	2.1
XXVIII	.....	.....	.....	.....	.....	.....	.....	.....	0
XXIX	.....	.....	.....	.....	.....	.....	.....	.....	0
XXX	.....	.....	.....	.....	.....	.....	.....	.....	0
XXXI	.....	.....	.....	.....	.....	.....	.....	.....	0
XXXII	.....	.....	.....	.....	.....	.....	.....	.....	0
XXXIII	.....	.....	.....	.....	.....	.....	.....	.....	0
XXXIV	.....	.....	.....	.....	.....	.....	.....	.....	0
XXXV	.....	.....	.....	.....	.....	.....	.....	.....	0
XXXVI	.....	.....	.....	.....	.....	.....	.....	.....	0
XXXVII	.....	.....	.....	.....	.....	.....	.....	.....	0
Total	246.3	269.3	240.7	245.9	237.8	237.2	246.1	313.1	281.6

These graphs give further evidence on the nature of the growth of new shoots on the pear tree.

In case the total amount of growth produced on the mother shoots were dependent upon mere accidents of position or other factors of pure chance, we should expect to find that its frequency distribution would approximate the normal curve of errors.

In both cases the frequency distributions depart rather widely from that of the normal curve of errors. The values for the growth on the pruned shoots were found to belong to Pearson's type IV curve of frequency distribution in Rhind (*12*), and those of the unpruned shoots to a type I curve. Both distributions are distinctly skew toward the higher values

and indicate that there is a tendency in the population as a whole to produce more than the mean quantity of wood. This tendency in the growth response may, however, be opposed by some factor in the tree which operates to cut down the number of individuals attaining greater length.

TABLE II.—*Mean length of laterals on unpruned mother shoots*

Lateral No.	On mother shoots which had—		
	12 to 19 buds.	20 to 27 buds.	28 to 36 buds.
	Cm.	*Cm.*	*Cm.*
Apical	70.3	66.1	66.3
I	18.8	9.1	28.0
II	18.7	20.3	27.3
III	6.9	4.9	6.9
IV	4.5	4.4	1.1
V	4.0	2.8	3.4
VI	3.0	1.4	1.3
VII	1.8	2.7	1.0
VIII	1.9	.9	4.0
IX	3.9	1.3	1.3
X	3.7	2.5	1.4
XI	1.8	2.7	1.7
XII	1.4	1.5	7.3
XIII	.7	3.4	1.4
XIV	.6	1.8	3.7
XV	.6	1.5	1.6
XVI	0	.8	1.9
XVII	0	2.0	1.3
XVIII	0	1.6	2.4
XIX	0	.7	2.9
XX	.....	.5	1.4
XXI	.....	.3	.7
XXII	.....	.4	.7
XXIII	.....	.2	1.4
XXIV	.....	.2	1.0
XXV	.....	.1	.4
XXVI	.....	.0	.4
XXVII	.....	.0	.3
XXVIII	.....	.....	0
XXIX	.....	.....	0
XXX	.....	.....	0
XXXI	.....	.....	0
XXXII	.....	.....	0
XXXIII	.....	.....	0
XXXIV	.....	.....	0
XXXV	.....	.....	0
XXXVI	.....	.....	0
Total	142.6	134.1	172.5

To attempt any far-reaching analysis of the causes of the greater range and variability in the shoot production on the pruned trees would be unprofitable with nothing more than the data at hand. There is evidence that the orientation of a shoot with respect to a perpendicular line has a controlling influence per se upon the amount and distribution of subsequent growth. In selecting the material for this study the at-

tempt was made, however, to avoid all shoots which to any measureable extent departed from the perpendicular. Further experimental work ought to be done to determine the ratio between the new material produced and the amount of shoot pruned off, as well as the ratio between the amount of new material produced and that left on the tree.

At this point the question may logically be asked, "Does the severity of the pruning affect the total amount of wood produced in the following season?" To answer the question we may consult the data given in Table III, which sets forth the mean total length of laterals produced on pruned and unpruned mother shoots. On the pruned trees the class range is three buds, on the unpruned it is eight buds. The larger range in the latter case is due to the smaller population and the necessity of avoiding classes of very low frequencies.

TABLE III.—*Total production of laterals on mother shoots possessing varying number of buds*

Mean number of buds on mother shoots.	Treatment of mother shoots.	Total mean lateral production.	Root-mean-square deviation.
		Cm.	
5	Pruned	246.3	
8	do	269.3	
11	do	240.7	
14	do	245.9	
17	do	237.8	
20	do	237.2	
23	do	246.1	
26	do	313.1	
32	do	281.6	
Mean		257.6	24.22
16	Unpruned	142.6	
24	do	134.1	
32	do	172.5	
Mean		149.7	16.47

It is easily seen from Table III that there is little real difference in the mean total length of laterals on mother shoots of different sizes. In both pruned and unpruned mother shoots it will be seen that, so far as total shoot growth is concerned, there is no marked or consistent difference between the various classes, though there is a tendency for greater growth output on the class of mother shoots possessing from 25 to 27 buds. It will be found, however, that the values are not so widely dispersed from their respective means as to indicate deviations of marked biological significance. In each case the deviation of the

several values from their mean is only about 10 per cent of the mean. In dealing with biological material we should not be justified in attaching any significance to such slight differences, since they might just as probably be due to factors which produce chance variations.

It therefore seems evident from these figures that the growth output of a mother shoot is practically independent of the number of buds it possessed or of the number of laterals it produced. On the mother shoots on the pruned trees the largest number of laterals in the various classes ranged from 4 to 36, nevertheless the total production of laterals was remarkably uniform, considering the variability commonly encountered in biological material. It should be noted that the differences in length of the pruned shoots were due to the varying amounts of wood removed when they were pruned and not to inherent differences in their size.

These results seem to correspond with the data obtained by Miss Brenchley (*1*) on the total dry-weight production of barley and mustard in pots containing uniform quantities of soil. She found that, so far as total dry weight was concerned, there was no real difference whether there were 1, 2, 3, 4, or 5 plants per pot.

In this inquiry we have so far neglected one question which the reader is likely to raise, "Is there any tendency toward compensatory growth following the amputation of various amounts of the mother shoots?" In the category of biological ideas there has been some sort of idea that an injured organism, or one of its members, has a tendency in its growth to "restore lost parts." This idea, though often expressed in crudely anthropomorphic phraseology, has dominated many purely qualitative studies in regeneration. It is deserving of study in a quantitative way if possible.

The total new growth plus the length of the mother shoots may be compared for the different classes of mother shoots. Table IV gives the means of these various sums and the root-mean-square deviation of the series from their respective averages. For the pruned shoots the values range from 260.0 cm. for the "5" class to 381.7 cm. for the "26" class, a difference between extremes of 121.7 cm. The mean total lengths of laterals alone (Table III) had an extreme difference of 75.9 cm. The wider range of values in Table IV is to be attributed to the inclusion of the length of the mother shoots. Another way of showing the same relation is by the value of the root-mean-square deviation which measures the dispersion of the variates from their mean value. In Table III the root-mean-square deviations for the pruned and unpruned shoots were 9.4 and 11.0 per cent of their respective means. In Table IV these deviations were raised to 12.8 and 15.1 per cent of the respective means.

TABLE IV.—*Length of mother shoots plus total length of new wood produced on them*

Mean number of buds on mother shoot.	Treatment of mother shoots.	Length of mother shoot plus total new growth.	Root-mean-square deviation from mean.
		Cm.	
5	Pruned	260.0±14.6	
8	do	293.1±12.6	
11	do	276.5± 8.2	
14	do	288.7±11.6	
17	do	285.0±12.3	
20	do	283.6±11.7	
23	do	307.2±16.1	
26	do	381.7±24.6	
32	do	366.3±23.9	
Mean		304.7±8.79	39.08
16	Unpruned	178.6±10.8	
24	do	185.4± 6.5	
32	do	247.1±14.9	
Mean		203.7±12.0	30.81

The coefficient of correlation between the length of the mother shoots and the total amount of new growth produced by them is a more concise way of expressing the degree of association between the two variables. The values of these coefficients were found to be—

For the unpruned shoots.....$r=0.140\pm0.074$.
For the pruned shoots.....$r=0.004\pm0.041$.

The first coefficient is only twice its probable error and can not be regarded as of much significance, and the second coefficient fails to show any degree of association whatever. It does not appear, therefore, that there is any tendency in these pear trees toward stabilizing the size of the system, mother shoot plus new laterals. In other words, there is no tendency toward a "restoration of lost parts" through growth.

The absence of such a tendency to restore lost parts is also emphasized by the amount of growth produced on mother shoots which received no pruning. Without any stimulus of that sort they produced 58 per cent as much new growth as their pruned neighbors.

RELATIONS BETWEEN THE SIZE AND THE POSITION OF LATERALS ON THE MOTHER SHOOTS

The laterals growing from the buds on a mother shoot exhibit differences which are so characteristic as to attract the attention of the most casual observer. On the pear, as well as other trees, the longest lateral is usually produced at the apex of an upright mother shoot, and each successively lower lateral is usually shorter than the one above it. We may proceed to a study of the quantitative relations of the laterals in this population.

The mean length of the laterals is given in Tables I and II, but it will facilitate the inquiry to have them assembled in basipetal order as in Table V. These means are composites of those in Tables I and II, but in making them due weight was given to the shoot-producing power of each class of mother shoots. The shorter mother shoots were necessarily omitted in making up the means of buds having higher serial numbers. For example, consider a mother shoot which had, after pruning, 14 buds. Obviously this shoot could produce no laterals from the twentieth bud, because it had none. Therefore, it was omitted from all classes having more buds than 14 at the outset. If a mother shoot possessing originally 26 buds failed to produce a lateral from the twentieth bud, its production (for that bud) was counted as zero and the mean was correspondingly influenced.

TABLE V.—*Mean length of laterals produced on mother shoots*

Lateral No.	Pruned.		Unpruned.	
	Observed.	S.	Observed.	S.
	Cm.	*Cm.*	*Cm.*	*Cm.*
Apical	.....	.....	67.76	67.76
I	110.18	110.18	15.49	44.22
II	69.14	70.66	20.67	10.72
III	31.14	40.09	5.94	12.34
IV	11.03	20.08	4.01	4.64
V	9.01	6.43	3.34	3.01
VI	1.82	6.29	2.01	2.74
VII	3.57	1.93	2.13	2.29
VIII	2.04	2.23	2.56	2.22
IX	.88	1.64	2.31	2.69
X	1.23	1.51	2.82	2.27
XI	2.14	2.40	2.22	2.52
XII	3.57	1.85	2.21	2.16
XIII	1.55	2.38	2.09	1.90
XIV	1.18	1.05	1.58	1.63
XV	.55	1.53	1.16	1.11
XVI	1.87	1.44	.63	1.15
XVII	2.32	1.44	1.13	.86
XVIII	1.01	2.53	1.08	.92
XIX	2.73	.62	.71	.89
XX	.22	1.71	.69	.60
XXI	.68	1.06	.48	.53
XXII	1.89	1.77	.37	.44
XXIII	2.85	2.88	.39	.33
XXIV	3.87	2.69	.29	.30
XXV	2.33	3.04	.22	.25
XXVI	2.21	1.52	.21	.22
XXVII	.71	1.11	.22	.11
XXVIII	0	.36	0	.11
XXIX	0	0	0	0
XXX	0	0	0	0
XXXI	0	0	0	0
XXXII	0	0	0	0
XXXIII	0	0	0	0
XXXIV	0	0	0	0
XXXV	0	0	0	0
XXXVI	0	0	0	0

The data presented in Table V show that the lengths of the laterals arranged in basipetal order form some sort of a descending series. The lengths of the first half dozen laterals descend very rapidly; indeed, from that point down there is no marked decrease in values in the pruned series, though the unpruned series shows a second descent from about lateral XVIII. In data of this kind it is frequently advantageous to use S instead of the observed values of y. In this case S is computed from

$$\frac{y_{x-1}+y_{x+1}}{2},$$

FIG. 3.—Logarithmic values of S (the adjusted value of the mean length of the laterals). The solid line indicates the values for the laterals on pruned mother shoots and the broken line the values for the laterals on unpruned mother shoots.

when y represents length of the lateral and x represents its ordinal position on the mother shoot. Thus the value of S for any given lateral represents the average of the preceding and the following laterals. As McEwen and Michael (*6*) have pointed out, this takes advantage of the fact that the slope of a chord of a simple curve is approximately equal to the slope of the tangent at the point midway between the extremities of the chord. Its use enables one to smooth out inequalities due to errors of sampling or to other causes. In comparing the various values of S it is convenient to employ logarithms of the values, as was done in making the graphs shown in figure 3, because of the wide range of the figures and of the difficulty in using a satisfactory scale. It is obvious that these graphs would descend in a comparatively straight

line from left to right if the lengths of the laterals formed a regularly descending geometrical series. As mentioned above, the descent is by no means uniform. The logarithms of the first seven values of S descend rapidly and somewhat uniformly, but from that point on the descent is less rapid and more irregular. In the language of the horticulturist, the first few buds formed shoots and the rest formed fruit spurs. The formation of these two classes of laterals is of great importance in horticultural practice, and their regulation is one of the principal objects of pruning. A discussion of the physiological aspects of the problem will, however, be postponed until other data have been presented.

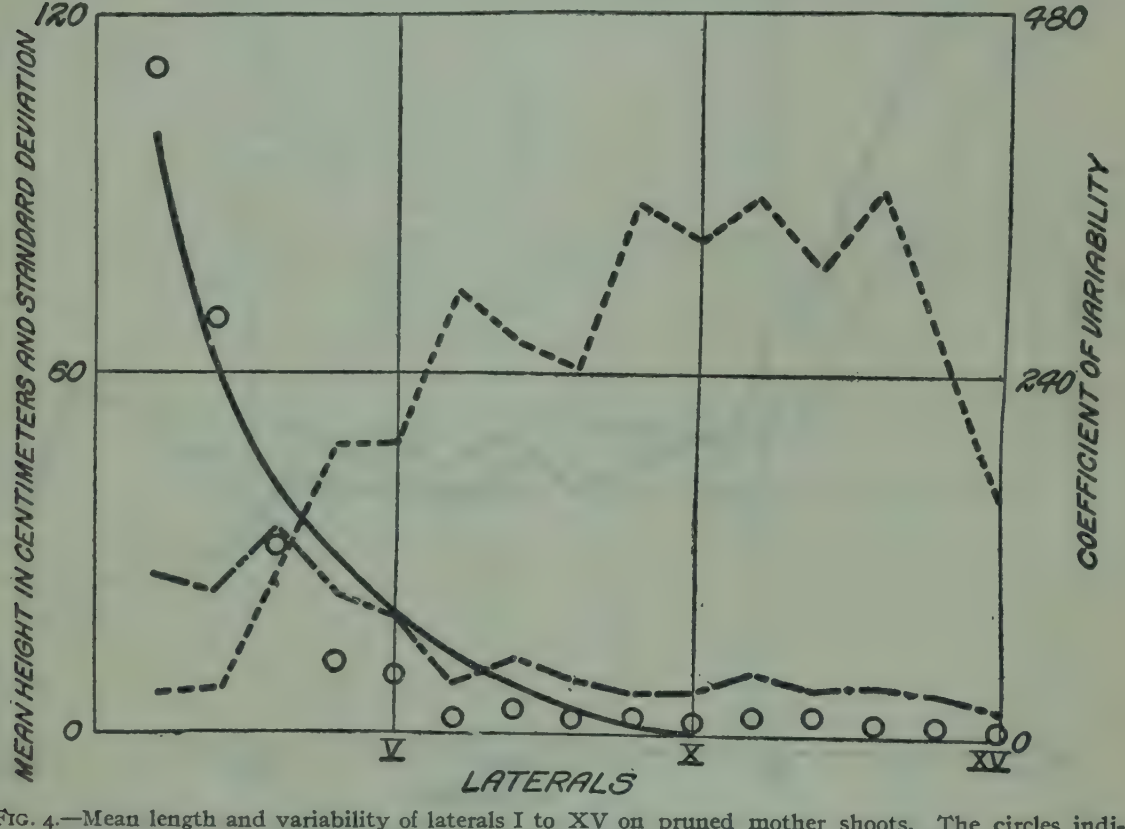

FIG. 4.—Mean length and variability of laterals I to XV on pruned mother shoots. The circles indicate the observed mean lengths of laterals; the dots and dashes, the standard deviation; the broken line, the coefficient of variability of laterals; and the solid line, the mean length of laterals I to X calculated from $y=93.47+4.193x-136.907 \log x$.

The length of the first 10 laterals when plotted as in figure 4 indicates that they follow a curve of a type frequently encountered in biological data. By the aid of tables given by Pearl and McPheeters (*8*) the equation for this curve was computed and found to be

$$y=93.47+4.193x-136.907 \log x,$$

when y represents length of lateral and x represents its ordinal position on the mother shoot. The path of this curve is shown in figure 4. That it is more representative of the laterals than of the fruit spurs is shown both by its shape and by the fact that it gives values of zero or less from lateral X and below.

These figures clearly betoken certain biological relationships which must be of prime importance in attempting to understand the laws of development in higher organisms. They make it possible to extend the ideas on integration and differentiation which formed such a considerable part of Herbert Spencer's (*13*) "Principles of Biology." The figures presented show that the length of a lateral is some sort of a function of its ordinal position on the branch which bore it. That the length of a given lateral is as much an expression of the properties of the branch as the angles of a crystal are of the properties of a salt there can be no reason to doubt. This is not to say that the equation giving the length of a lateral will necessarily disclose the physiological factors determining it, but it does banish the idea that form and function are so variable as to be outside of the realms of exact science.

The series of correlations reported on a subsequent page are further evidence of a functional relationship between the various laterals with due regard to the factors which may reside in the mother shoot. The representation of their relative length by a mathematical equation is not, therefore, due to chance or to the fortunate choice of an equation. Future work in this important field on subjects such as those investigated by Pearl (*7*) and by Johnson (*4*) should afford information of the utmost value.

VARIABILITY IN THE LENGTH OF LATERALS

The biological constitution of the population under consideration was next investigated. The nature and amount of variability existing in the population was used as an indicator of its genetic character. The laterals on the pruned shoots served as a basis for the inquiry, because of the larger number in the population.

A graph in figure 4 shows the coefficient of variability of the first 15 laterals produced on the pruned mother shoots. The coefficients of variability of these means increases somewhat irregularly from 23.18 for lateral I to 359.90 for lateral XIII and falls to 159.52 for lateral XV. This indicates that the laterals produced from the distal end of the mother shoot are less variable in length than those produced nearer the proximal end of the shoot. This increased variability can not be attributed to the smallness of the group or to a poor sample, because lateral XIII comprised a population of 172 individuals.

The fact that minimum variability occurs in the members at the distal end of the shoot has previously been noted in Ceratophyllum (*7*). On Ceratophyllum the whorls of leaves borne at the distal end of the shoot may be considerably younger than those borne at the opposite end. On the pear shoots the distal lateral is likely to be the oldest on the mother shoot, though in many cases the difference in age is very slight. The position of the lateral upon the parent axis appears to be more of a factor than age in determining variability in length.

Another phase of the variability of the laterals is shown by the graphs in figure 5, which show the frequency distributions of laterals I and V in the pruned class and of the apical shoot in the unpruned class. The graphs for the apical lateral in the unpruned class and for lateral I are fairly symmetrical but have a skewness in the direction of the higher values. The graph for the lateral V is very asymmetrical because a disproportionate number of variants fall in the upper classes. Each of these graphs indicates that the variability in the length of the laterals is not a chance phenomenon, but that it is a function of their position on the mother shoot. The tendency of these factors is to produce more long laterals in each class than would be found if their length were

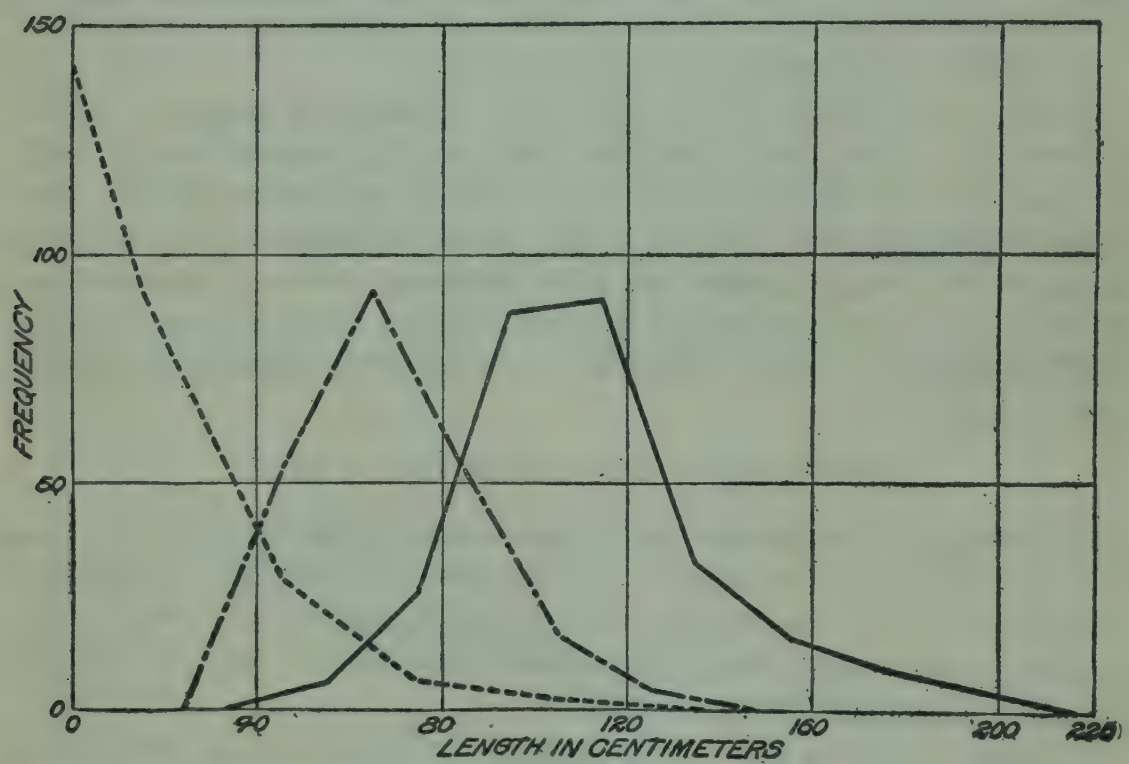

FIG. 5.—Frequency distributions of the mean lengths of certain laterals. The solid line represents lateral I on pruned mother shoots; the broken line, lateral V on pruned mother shoots; and the dots and dashes, the apical lateral on unpruned mother shoots.

determined by pure chance. That this influence is inherent in the tree and not due to the operation of pruning is shown by the same tendency in the frequency distribution of the laterals arising from the apical buds on the unpruned mother shoots.

II. DOMINANCE OF THE DISTAL LATERALS OF THE PEAR TREE

It is hardly necessary to mention again that the distal lateral on a vertical shoot is usually larger than any other lateral growing between it and the base of the mother shoot. It will be profitable to study the problem somewhat further with the hope of gaining more light on the causes of this characteristic type of growth.

An inspection of the tables giving the size of laterals on the mother shoots shows that, as a rule, the length of the distal lateral has some sort of inverse ratio to the length of its mother shoot. The relationship was determined by computing the coefficient of correlation between

the length of the distal laterals and the number of buds possessed by the mother shoots upon which each was produced. The number of buds is thought to be of greater biological significance in this case than the actual length of the mother shoot. The value of this coefficient for the pruned shoots is $r = -0.266 \pm 0.038$, and for the unpruned -0.196 ± 0.088. The negative coefficients are in harmony with the relations already observed between mother shoots and their distal laterals. The coefficient expressing the negative correlation between the length of the pruned mother shoots and their distal laterals is statistically significant and indicates that the more severely the mother shoots were pruned

FIG. 6.—Regression of mean length of distal laterals on mean number of buds per mother shoot. Circles indicate observed mean length of laterals on pruned mother shoots; the solid line, the curve of means calculated for laterals on pruned shoots; the triangles, the observed mean length of laterals on unpruned mother shoots; and the broken line, the curve of means calculated for laterals on unpruned mother shoots.

the longer were their distal laterals. For the unpruned mother shoots the correlation coefficient is likewise negative, but statistically less reliable because of the relatively large probable error accompanying it. Had the calculations been based upon a larger population, this coefficient might have been closer to that for the other class of shoots.

The regression of the means in the two cases is shown in figure 6. The calculated values were obtained from the formula

$$X = \left(\bar{x} - r\frac{\sigma_x}{\sigma_y}\bar{y}\right) + \left(r\frac{\sigma_x}{\sigma_y}\right)y,$$

where $\bar{x}$ is the mean value of the x's, $\bar{y}$ of the y's, and r is the correlation coefficient.

We may digress momentarily to see whether the pruning increases the dominance of the laterals produced in the distal region of the mother shoot. For example, in Table I it will be seen that the class of mother shoots having 23 original buds produced a mean total shoot growth of 242.5 cm. and that the five upper buds produced 90.7 per cent of the total shoot wood. By comparing this with unpruned mother shoots having 24 original buds (Table II), it will appear that the five upper buds produced only 78.8 per cent of the total shoot growth. It will also be noted that 21 laterals were produced on the average pruned mother shoot mentioned, and 25 laterals were produced on the average unpruned mother shoot selected as an example.

A consideration of the relative length of the laterals on the mother shoots prompts one to determine the nature and amount of correlation existing between the laterals produced near the distal end of the mother shoots. The results of such an inquiry might be expected to throw some light upon two important questions—(1) "Is there a group of factors which tends to differentiate the mother shoots in their capacity for the production of laterals?" If so, they should find expression in a correlation between any two laterals on the same mother shoot. (2) "Are there factors which tend to differentiate the laterals on the same axis?" If so, they should find expression in differences in the correlation between laterals in different positions.

The coefficients given in Table VI show the correlation existing between the first four laterals on the pruned mother shoots when compared in different ways. The coefficients of ordinary, or gross, correlations are given at the head of each column. Below them stand the coefficients of partial correlation.

The latter coefficients are intended to express adequately the amount of correlation existing between nonadjacent shoots. They are an attempt to answer such a question as, "What is the correlation between the first and third laterals, assuming that all the second laterals were of equal length?"

TABLE VI.—*Coefficients of gross and partial correlation of the length of the four distal laterals on pruned mother shoots*

r_{12} ..... 0.346±0.036	r_{13} ..... 0.296±0.037	r_{14} ..... 0.157±0.040
$r_{12.3}$ ..... .266± .038	$r_{13.2}$ ..... .211± .039	$r_{14.2}$ ..... .090± .041
$r_{12.4}$ ..... .324± .037	$r_{13.4}$ ..... .267± .038	$r_{14.3}$ ..... .083± .041
$r_{12.34}$ ..... .258± .038	$r_{13.24}$ ..... .197± .039	$r_{14.23}$ ..... .046± .041
r_{23} ..... 0.313±0.037	r_{24} ..... 0.216±0.039	r_{34} ..... 0.272±0.038
$r_{23.1}$ ..... .235± .039	$r_{24.1}$ ..... .175± .040	$r_{34.1}$ ..... .239± .039
$r_{23.4}$ ..... .270± .038	$r_{24.3}$ ..... .143± .040	$r_{34.2}$ ..... .221± .039
$r_{23.14}$ ..... .202± .039	$r_{24.13}$ ..... .126± .040	$r_{34.12}$ ..... .207± .039

The coefficients of gross correlation are all positive and of sufficient magnitude in comparison with their probable errors to be considered significant. They indicate that the growth of one lateral is correlated

with the growth of other laterals on the same mother shoot and that the correlation between adjacent laterals is greater than for nonadjacent laterals, suggesting that the factors determining the amount of growth are not uniformly distributed in the mother shoots. Moreover, the greatest correlation is found between laterals at the distal end of the mother shoots and becomes less with each successively lower pair of laterals.

The coefficients of partial correlation go somewhat further in showing these relations. A comparison of r_{12}, $r_{12.3}$, and $r_{12.4}$ shows that by equalizing the effect of the third lateral, the coefficient is diminished from 0.346 to 0.266, but equalizing the effect of the fourth lateral reduces it only from 0.346 to 0.324. Broadly speaking, the length of the fourth lateral has less effect than that of the third upon the correlation between the length of the first and second laterals. When the length of both the third and fourth laterals is equalized the correlation between the first and second laterals is reduced from 0.346 to 0.258. The value of r_{14} is the lowest of all the gross correlations and the coefficients of partial correlation into which r_{14} enters are so low in comparison with their probable errors as to lack statistical significance.

The values of these coefficients may, therefore, be regarded as good evidence in favor of an integral relationship in the growth processes of neighboring laterals on an upright mother shoot and that neighboring laterals have a tendency to vary together. It would seem difficult to escape the conclusion that factors which promote growth in any lateral also tend to promote growth in the adjacent lateral. It would at least seem that these measurements speak against any explanation which rests upon the assumption that the dominant apical shoot is such because it has withdrawn food or growth stimulators from the subapical region of the mother shoot. It is quite possible that two distinct groups of factors are operating to influence the growth of these laterals, one of which tends to make all the laterals of a given mother shoot either longer or shorter than the average for the population as a whole, while the other group tends to oppose the development of the subapical shoots, this suppression being the more complete as the distance from the distal end of the shoot increases. The existence of significant positive correlations between adjacent laterals speaks for the existence of the first group of factors. The systematic differences in the values of the interlateral correlation coefficients speak in favor of the second group of factors.

This may mean that while there is a well-marked tendency for the factors which produce length in the first lateral to produce length in the laterals below it, there is some other factor at work which tends to block the operation of the length-producing factor in the subapical shoots and that it is more completely blocked as one proceeds down the axis of the mother shoot.

Further light on these questions may be obtained if we inquire concerning the possibility that any lateral may grow to the length of the lateral immediately above it. By use of a formula given by Harris (*3*) we may obtain a quantitative expression of this capacity of subapical laterals to come to development. The capacity of the second lateral to develop with respect to the first might be expressed as

$$r_{xz} = \frac{r_{xy} - \frac{V_x}{V_y}}{\sqrt{1 - r^2_{xy} + \left(r_{xy} - \frac{V_x}{V_y}\right)^2}},$$

where r_{xy} is the coefficient of correlation between the two characters and V_x and V_y are their coefficients of variability.

Determination of these constants gives the following:

Lateral II with respect to lateral I -0.883 ± 0.009.

Lateral III with respect to lateral II -0.876 ± 0.010.

Lateral IV with respect to lateral III -0.830 ± 0.013.

Lateral III with respect to lateral I -0.973 ± 0.002.

Lateral IV with respect to lateral II -0.958 ± 0.003.

Lateral IV with respect to lateral I -0.991 ± 0.001.

These coefficients measuring the correlation between the length of the subtending lateral and the deviation of the variables from their probable values are all negative and relatively large. Their values have a tendency to increase as the distance between the laterals increased.

The results show that the subtending laterals fail to attain the size we should expect when the more distal laterals have a larger size. In short, there is the clearest evidence of a competition, or of an inhibition of growth, associated with the development of the higher laterals.

III. PRODUCTION OF FRUIT SPURS

The short, spur-like branches on the limbs of the pear and other trees call for special consideration. They differ from the longer pliable shoots of the same tree in being primarily fruit-bearing shoots and, in the parlance of the horticulturist, are called "fruit spurs." As is well known, they grow slowly in length, have short internodes, and characteristically bear a flower bud at the apex instead of a vegetative bud. The fundamental physiological and morphological differences between the two classes of shoots have been clearly set forth by Vöchting (*14*) and others.

If we grant that the production of fruit spurs, as well as that of the longer laterals, is a function of the growth process of the tree, we may proceed to inquire concerning their distribution and the laws of their characteristic growth. The difference between fruit spurs and purely vegetative shoots, as mentioned above, is primarily one of degree and not of kind. Consequently it is necessary to fix, arbitrarily, the length

limit which shall define a fruit spur. In this investigation of young branches the limit has been set at 5 cm., and, in accordance, all shoots having a length of 5 cm. or less are designated as fruit spurs.

The number of fruit spurs on the pruned mother shoots ranged from 0 to 13. The larger number were naturally found on the longer mother shoots. The energy of the shorter mother shoots appears to have been used mainly for the production of vegetative shoots. The number of fruit spurs on the unpruned mother shoots ranged from 1 to 20, but only two shoots had fewer than 4 fruit spurs.

The data given in Table VII show the mean number of fruit spurs produced on mother shoots of various classes. The mean spur production of the shorter pruned mother shoots is very low, but increases, somewhat irregularly, until the largest number is found on the mother shoots having more than 28 buds. There is a well-marked tendency, however, to less variability in spur formation on the longer mother shoots. The last two classes of mother shoots on the pruned trees seem to be in a class somewhat different from the others in respect both to the mean number of fruit spurs formed and in having much less variability with respect to the mean. It would seem that these pruned mother shoots failed to produce more than a minimum of fruit spurs except when they possessed more than 25 buds per mother shoot.

The mother shoots on the unpruned trees produced considerably more fruit spurs than their pruned neighbors. The lowest average in this group was practically equivalent to the highest average among the pruned trees. The variability in the number of fruit spurs with respect to their mean was less in the longest mother shoots than in those of any other class. Clearly there is a manifest tendency for decreased variability to be associated with increased production of fruit spurs. This characteristic may also be discerned in the growth of vegetative laterals at least so far as their length is concerned. The greatest output of growth material leads to the production of the longest laterals and to the lowest variability.

TABLE VII.—*Variability in the production of fruit spurs on mother shoots*

Number of original buds on mother shoot.	Treatment of mother shoot.	Mean number of fruit spurs per mother shoot.	Coefficient of variability.
4 to 6	Pruned	0.75±0.20	135.71±40.38
7 to 9	do	.78± .13	142.31±27.00
10 to 12	do	2.53± .18	78.26±10.82
13 to 15	do	2.23± .37	163.23±29.82
16 to 18	do	3.60± .34	93.89±11.07
19 to 21	do	2.52± .29	98.41±13.97
22 to 24	do	2.67± .41	119.10±21.42
25 to 27	do	5.07± .51	57.79± 9.24
28 and more	do	7.88± .69	38.83± 7.47
12 to 19	Unpruned	7.33±1.52	141.40±32.83
20 to 27	do	12.15± .64	40.00± 4.32
28 to 36	do	15.86±1.14	28.32± 5.48

The graphs in figure 7 show the distribution of fruit spurs on two classes of mother shoots, one of which was typical of the pruned and the other of the unpruned shoots. They show that the types of distribution in the two cases are fundamentally different. For the pruned shoots the greatest frequencies were observed for those having 3 fruit spurs or less. For the unpruned mother shoots there were none having less than 4 fruit spurs, and the highest frequencies were found for shoots having 12 to 18 fruit spurs.

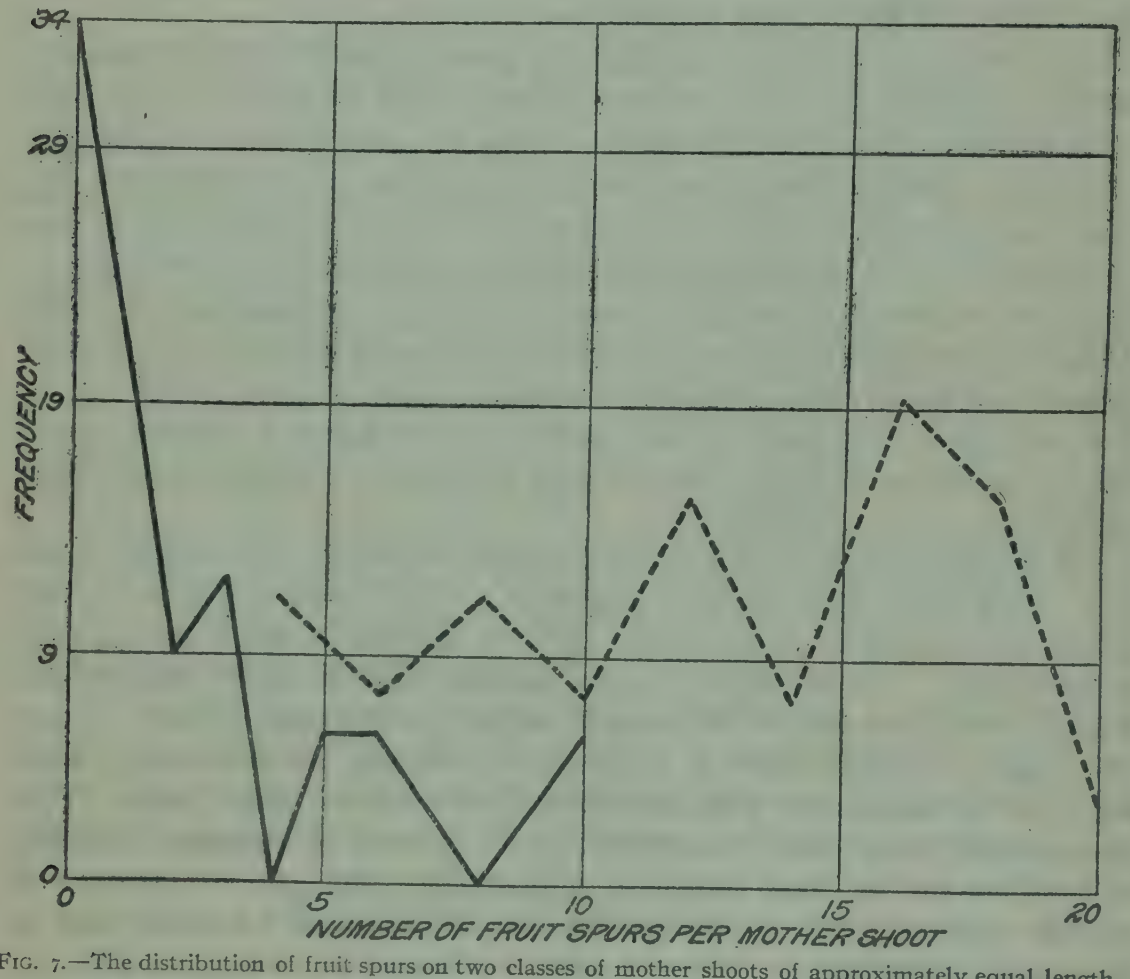

FIG. 7.—The distribution of fruit spurs on two classes of mother shoots of approximately equal length. (Frequencies are expressed as percentages of *n*.) The solid line indicates the distribution on principal mother shoots having 19, 20, or 21 buds and the broken line the distribution on unpruned mother shoots having 20 to 27 buds.

We may say, therefore, that the typical pruned mother shoot was characterized by few fruit spurs and that the typical unpruned mother shoot was characterized by relatively many fruit spurs.

The foregoing statements may appear to contain nothing especially new to those familiar with the art of pruning. They do furnish, however, quantitative data upon which an analysis of the growth response of the tree may be based. Horticulturists have long known that little or no pruning results in the production of shorter laterals and of more blossoms. Whether the tree is thereby rendered more productive or whether the fruit is of satisfactory quality is, however, another question.

DISCUSSION

It remains to see how the data presented above will contribute to an understanding of the growth process in trees, especially in relation to the characteristic formation of vegetative and fruit-bearing shoots. It hardly requires further discussion to show that the new shoots on the pear tree arise in such a perfectly definite and orderly fashion as to suggest that their growth and distribution is governed by definite causal factors. The problem is to discover, if possible, these causal factors and their functions. The discussion will adhere in a general way to the arrangement of data presented in the preceding pages.

The total amount of new shoot material produced on pruned and unpruned mother shoots ought to show what result the stimulus of pruning may produce, and to give a basis for discussing the nature of accelerated growth.

A comparison of the amount of new growth produced in the two cases shows that the branch system is larger at the end of the season than would be required if the growth response were merely a means of restoring what had been removed by pruning. In other words, the amputation of part of the mother shoot has resulted in producing more new growth on what remained of that particular shoot than would have been produced if it had remained uncut. The problem is not, why such a shoot produces new laterals, but why it produces a mass of new laterals about 65 per cent greater than it would if left unpruned. Since this problem is fundamental to the theory and practice of tree pruning, it may be profitable to extend the inquiry as far as possible.

The simplest conception of growth which we seem warranted at present in holding is that it is the result of some sort of a catalyst acting upon substances acquired by, or produced in, the organism. In the absence of contrary evidence we must believe that all buds and other meristematic tissues are supplied with either the active or the potential catalyst. The ratio between the growth of a particular organ and the growth of the rest of the tree must in some way depend upon the relative activity of the catalyst of these organs.

In connection with these suggestions reference may be made to a paper on the growth of pruned and unpruned branches of the apricot tree (*9*). As a result of the pruning the final mean length of new shoots was 210 cm., while it was 94 cm. on the unpruned neighboring trees. The equation for the growth of these apricot shoots was that of a monomolecular chemical reaction,

$$x = a\ (1 - e^{-kt}),$$

in which x represents the length of the shoot at time t, a is the final length of the shoot, k is a constant, and e is the base of the natural logarithms. Having the values of a and k, it is possible to compute the length of the shoots for any value of t in the growing season. For these apricot shoots

the value of k was the same, but that of a differed in the two equations. The rate of growth in such a case is expressed by the differential equation

$$\frac{dx}{dt} = k\ (a - x)$$

or,

$$\frac{dx}{dt} = k\ a\ e^{-kt}.$$

The rate of growth of the shoot appears, therefore, to be dependent upon its final length. The rate may be affected by (1) a variation in the activity of the catalyst or (2) a variation in the supply of potential growth materials. The case of the apricot shoots is believed to be applicable to the pear shoots.

It is difficult to understand how the amputation of a greater or less portion of a mother shoot can increase the supply of potential growth material for the same except as it decreases the number of meristematic centers. Two arguments against the validity of such an assumption at once arise. In the first place, mother shoots which had been pruned produced more new material than unpruned mother shoots possessing the same number of buds (Table III). In that case the number of competing growth centers was the same, but the results were different. In the second place it has already been shown that the number of buds left on a pruned mother shoot had very little effect upon the total amount of new shoot wood produced. The deviation of the values from their mean was less than 10 per cent of the mean.

We, therefore, seem to have reason to assume that the cause of the increased growth is to be referred to an increased activity of the growth catalyst. The reason for this increased activity will be discussed in connection with the difference in growth of the several laterals.

If no opposing factors were operative we should expect that each mature bud on the mother shoot would develop into a lateral shoot. The size of the laterals would be largely determined by the amount of energy which they were able to obtain from the parent tree supplemented by what they could obtain from outside sources. To state it somewhat more specifically, the size of a young lateral at the outset would depend upon the amount of growth-promoting material which the parent tree could furnish it. In course of time, however, the assimilatory system of the new shoot would be able to furnish additional supplies of material, a part of which could be used to further the growth of that shoot. The amounts of growth-promoting materials obtained in the latter, as well as in the former, case would largely be determined by the position of the shoot, which would in turn determine the conditions of competition for water, light, and other factors. The buds near the base of the mother shoot might, therefore, be expected to grow first into shoots and subsequently to develop as their access to light should give them opportunity.

The most casual observer is aware, however, that the lateral shoots arising from an older vertical shoot are by no means equal in length. The longest lateral is usually produced at the apex of the mother shoot, and each successive lateral is shorter than the preceding.

This characteristically greater growth of new shoots at the distal end of an upright branch, especially after pruning, is a well-recognized condition. An adequate understanding of its causes is greatly to be desired. Many of the statements on the subject to be found in botanical literature are vague, and others are mystifying. For example, the mere statement that "the available food materials of the stem are principally devoted to the development of the apical bud" raises more questions than it answers. The statement that the growth on an upright branch is regulated by polarity falls into the same category. In the sense that "polarity" has been applied to the formation of shoots and roots on cuttings this idea would be inapplicable to the factors influencing fruit-spur formation, since, in its implication, "polarity" involves the idea of two mutually exclusive, opposite qualities—for example, the opposite ends of a magnetized bar of metal. The idea of an "axial gradient" as used by Child (*2*) is a more exact designation of the phenomenon but does not account for the production of the gradient. If it be solely a question of the distribution of nutrient materials in the parent branch it is difficult to understand why the growing points at the distal extremity of the branch should acquire such quantities as would result in the production of larger shoots, while the growing points near the proximal extremity usually fail altogether to develop into shoots in the first season.

It seems logical to assume that the type of development observed depends not upon the distribution of nutrient materials in the parent branch but upon the distribution of some substance which is antagonistic to growth. This substance may be formed in the distal region of the shoot and migrate toward the base of the shoot. Certain evidence on this point has been already presented (*11*). We may, therefore, assume that the amputation of a portion of the mother shoot—removing the apical bud and a number of adjacent subapical buds—removed the most active centers in which some sort of a growth-inhibiting substance was produced. The effect of this operation was to remove an obstacle which impeded the development of subapical buds into shoots. This is an aspect of the theory of chalones which are recognized as factors in the growth of the animal body and which have been discussed in relation to plant growth by Loeb (*5*) and Reed and Halma (*10*, *11*). On the basis of this assumption it would appear that the buds immediately below the point of amputation grow first because they are the first to be freed from the inhibiting substance. As soon as the new shoots begin to grow they begin to produce more

of the inhibiting substance, which, in turn, tends to keep the buds below them dormant. The successively smaller size—diminishing length—of the lower shoots appears to be due to the effect of the growth-inhibiting substance produced in those above, an effect to which each shoot contributes, with the result that the lowest buds are entirely prevented from developing.

We may proceed to examine the plausibility of these theories as applied to the formation of laterals. On the supposition that a reduction in the length of the mother shoots so reduces the demands on the resources at the disposal of the tree that each growing point acquires a larger supply of these resources we should expect that the shorter mother shoots would produce more lateral growth than the longer mother shoots. Reference to the total lateral growth of the different mother shoots shows that this was not the case. If any difference existed it was in favor of the longer mother shoots. If we compare two classes of mother shoots originally possessing approximately the same number of buds, one of which was pruned and the other unpruned, we shall see that the pruned mother shoots produced considerably more total growth than the unpruned. A possible objection to this comparison may be mentioned—the shoots which were pruned were constitutionally more vigorous, because after removing a portion they were still as long as a certain class of unpruned mother shoots. An examination of Table III shows, however, that there is no ground for this objection, because the longest unpruned mother shoots failed to produce as much lateral growth as the shortest pruned shoots. The objection may, therefore, be dismissed.

An inspection of the trees in the orchard would convince any impartial observer that the pruned trees were in no way inferior in size to the unpruned, although the shape of the tree naturally differs. After three years the trees receiving heavy pruning each winter—the trees on which the pruned mother shoots were selected for measurement—had an average trunk girth of 16.2 ± 0.3 cm., and the unpruned trees had a girth of 16.6 ± 0.2 cm. Obviously the difference between the two is negligible.

Passing to the second possible explanation, that of an inhibitor, we should expect to find that the removal of the apical bud of a shoot, or of the zone in which the inhibitor was principally produced, would be shown by greater growth of laterals from the shoot remaining. So long as the principal source of the inhibitor were removed the total amount of new growth would have little, if any, relation to the length of the mother shoot until new supplies were produced. The data give strong evidence in favor of such an assumption. As soon as growth of the new lateral got underway more of the chalone would be produced and would inhibit growth in the subapical laterals so that total lateral growth would be limited and would diminish with the distance of the laterals from the apex of the mother shoot.

It would also appear that the data fail to support the assumption that in such cases a growth-stimulating substance migrates from the surrounding tissues and accumulates in the dominant area. It is quite probable that the growth-stimulating substances are of the nature of catalysts but that they occur in all buds on a mother shoot. We know that the amputation of a portion of a pear shoot will be followed by the growth of laterals from the buds immediately below the point of amputation (*10*). The data here presented show that the first laterals from the shorter mother shoots were longer than those from the longer mother shoots. Yet the latter had a larger surrounding area to draw upon if we assume that the growth of laterals was governed by the migration of growth-promoting substances. Furthermore, the slope of the regression line (fig. 6) indicates that the relationship between length of mother shoot and length of first lateral is negative throughout the whole population.

In order to remove a possible confusion in the mind of the reader, the substance of previous paragraphs may be repeated at this point. The shorter mother shoots, although producing longer apical laterals, nevertheless produced practically the same total of laterals as the longer mother shoots.

It would seem more logical to assume that each of the buds on the mother shoot is provided with the growth-stimulating substance (catalyst) and that all will grow provided they are sufficiently freed from substances which inhibit the action of the catalyst.

SUMMARY

(1) The paper contains an attempt to analyze and correlate the growth phenomena of young shoots on branches of the pear tree.

(2) Mother shoots which had been headed back in the previous winter produced about 65 per cent greater total growth of laterals than their unpruned neighbors. The frequency distributions of laterals on both classes of mother shoots show a positive skewness, which is taken to indicate a tendency to produce more than the mean quantity of wood.

(3) If mother shoots received any pruning whatsoever, the severity of the pruning seemed to have little influence upon the total amount of new laterals produced in the following season. The growth response showed little tendency toward a "restoration of lost parts."

(4) The longest lateral on a mother shoot is usually at the distal end, and each successively lower lateral is usually shorter than the one above it. The mathematical relationships of the system of laterals indicate a definite functional relation of the growth processes involved. Variability in the length of laterals tends to increase as their distance from the distal end of the mother shoot increases.

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(5) The lateral arising from the distal end of an upright mother shoot exerts a well-marked dominance over those arising from buds beneath it. The mother shoots which were most reduced in length by pruning usually produced longer distal laterals.

(6) There is a well-marked positive correlation in the length of neighboring laterals on the same mother shoot, but the correlation diminishes as the distance between the points of origin of the laterals increases; that is to say, the longer the distal lateral becomes the more will the subtending laterals fall short of the size which they might be expected to attain from the general size relations in the population under consideration. In harmony with such a relationship, it was found that the capacity of any lateral to reach the length of a lateral situated higher on the mother shoot is expressed by a relatively large negative coefficient.

(7) Fruit spurs were commonly more abundant upon unpruned mother shoots than upon those receiving pruning in the previous winter.

(8) The growth response indicates that increased growth following pruning is due to the removal of regions containing, or producing, substances which would otherwise tend to inhibit growth of other members of the system. Each mature bud on the mother shoot seems capable of developing into a lateral provided it be sufficiently free from growth-inhibiting substances.

(9) On the assumption that the pear branches produce a growth-inhibiting substance which flows in a basipetal direction, it is logical to find a gradient in the formation of laterals such as that existing here. The inhibitor would be more concentrated as the distance from the apex of the shoot increased. The effect of the increasing concentration would be to retard increasingly the growth of the lower laterals until finally such a concentration would be reached as to inhibit growth entirely. In this way the dominance of the apical shoots would be not only established but perpetuated.

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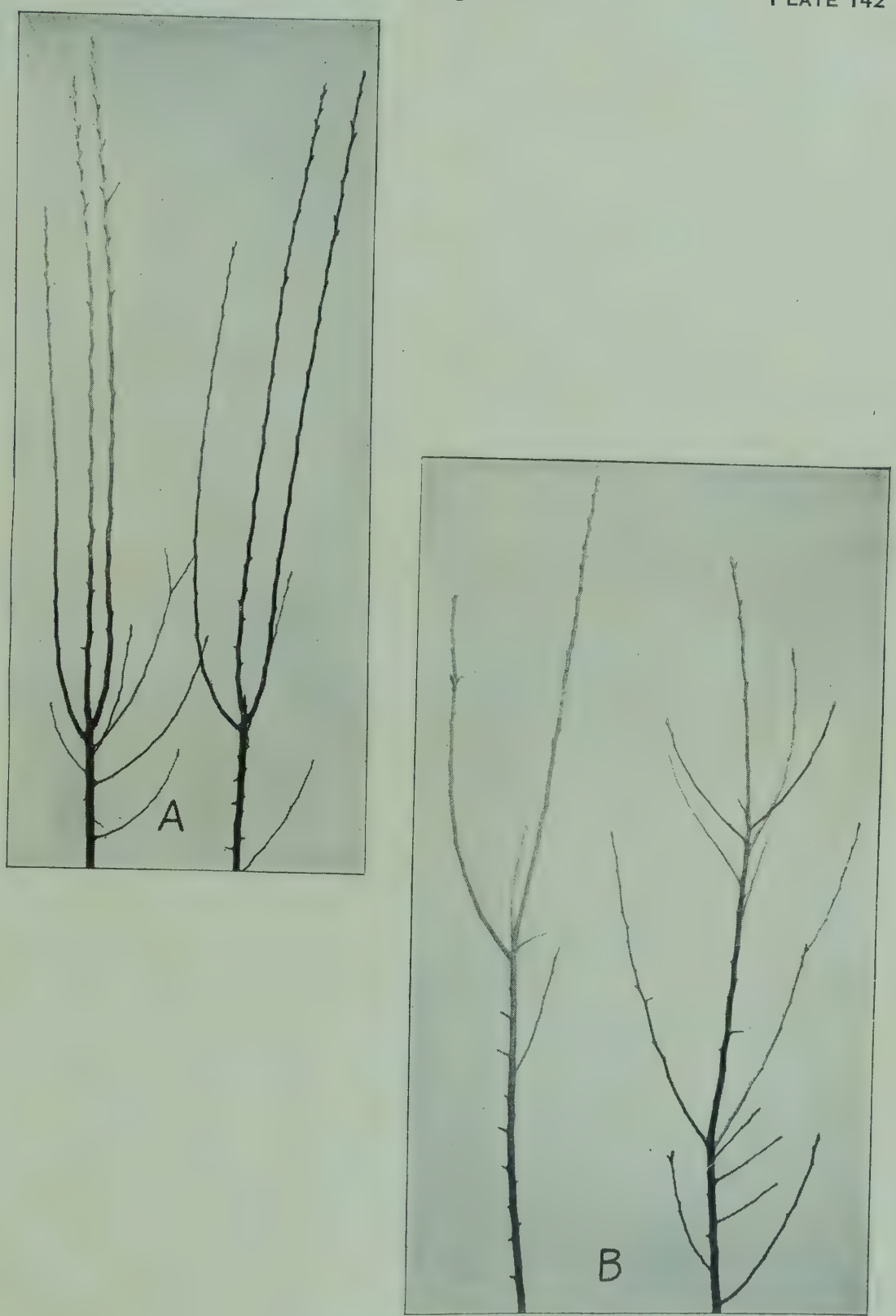
PLATE 142.

Examples of pruned and unpruned mother shoots with laterals at end of growing season.

A.—Pruned.

B.—Unpruned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